Genetic structure, diversity, and population ecology of Antarctic benthic amphipods

by

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Chapter 2 of this thesis has been published in a peer-reviewed journal:

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In both cases, the author **H. P. Baird** was responsible for the overall study design and implementation, the acquisition, analysis and interpretation of data, and the writing and final revision of the manuscript (≥85% of work). However, this work was assisted by the co-authors as outlined below.

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J. S. Stark assisted in idea formulation, sought logistical support for field work, and provided editorial revisions on the manuscript.

Chapter 3

K. J. Miller assisted in idea formulation, sampling design and sampling, sought financial support for lab work, provided guidance with data analysis and data interpretation, and provided editorial revisions on the manuscript.

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Abstract

With increasing anthropogenic threats to the marine environment, it has become a priority to improve our understanding and conservation of marine fauna. In Antarctic waters, a rich and diverse benthic fauna thrives. However, the relative isolation of these organisms and their adaptation to the unique Antarctic environment potentially heightens their vulnerability to environmental change. Thorough research on the genetic and ecological structure of Antarctic benthic invertebrate populations is lacking, particularly for some of the most dominant taxa, such as the Amphipoda. This study investigated genetic structure, diversity and population ecology in some common Antarctic benthic amphipod species, to build a more rigorous understanding of the Antarctic benthos that will aid in future management planning.

Genetic structure was explored over a circum-Antarctic scale in the widespread amphipod species *Eusirus perdentatus* and *Eusirus giganteus* from the continental shelf, using DNA sequences of two mitochondrial regions (COI and CytB) and one nuclear region (ITS2). Phylogenies and haplotype networks provided strong evidence that *E. perdentatus* harbours two previously undetected cryptic species, and *E. giganteus* harbours at least three, highlighting our current misunderstanding of Antarctic benthic diversity. There were clear differences in the distribution, genetic diversity and connectivity of populations within each cryptic species, and it is proposed that this reflects different modes of post-glacial recolonisation of the continental shelf. Within one cryptic species, high genetic population differentiation ($F_{ST} > 0.47$, $p < 0.01$) suggested a potential allopatric speciation process at play.

Genetic connectivity was explored over large (1000km) to very fine (100m) distances in the ubiquitous nearshore amphipod *Orchomenella franklini*, using seven highly polymorphic microsatellite markers. Genetic diversity differed significantly among populations, potentially reflecting local environmental conditions including anthropogenic pollution. Hierarchical AMOVA revealed marked genetic subdivision ($F_{ST} = 0.16$, $p < 0.001$) across the largest geographical scale and evolutionary isolation of these populations was inferred. Furthermore, three loci showed signs of selection across this scale, providing evidence of locally adapted populations. Gene flow was also

restricted at smaller scales, indicating a stepping-stone mode of dispersal consistent with the brooded development of amphipods.

The ecology of *O. franklini* was investigated through length measurements, sex and reproductive status of > 6000 individuals, spatial and temporal patterns in abundance, and corresponding relationships with environmental data. The life history of *O. franklini* revealed several traits that exemplify adaptation to the polar environment, including delayed reproduction, longevity (> 2 years), and seasonal breeding linked to the summer phytoplankton bloom. There was preliminary evidence of inter-annual and spatial fluctuations in reproductive timing, potentially reflecting local sea-ice conditions. *O. franklini* was found to reach astounding densities (> 65,000/m^2) and abundance was highly heterogeneous in space and time. The distribution of *O. franklini* was related to various sediment properties although the relationship differed with geographic region, highlighting a close association to the local environment as well as broader Antarctic conditions.

This study has provided significant insight into the dynamics of Antarctic benthic amphipod populations over a range of scales. Together the results emphasise a considerable degree of heterogeneity largely overlooked in Antarctic benthic organisms (from the taxonomy of entire species down to local-scale intraspecific population dynamics), and thereby support predictions of their vulnerability to anthropogenic-induced change. Results also shed light on speciation processes in Antarctic waters, and will ultimately help inform future planning decisions regarding spatial management of the Antarctic benthic ecosystem.

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Fieldwork & samples

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Chapter 1. General Introduction

1.1. ANTARCTIC BENTHOS

1.1.1. The Southern Ocean

The Southern Ocean surrounding Antarctica covers approximately 35 million km^2, accounting for almost 10% of the world's oceans by area, and exerting a strong influence on oceanographic and climatic patterns of the globe. The most prominent current in the Southern Ocean is the Antarctic Circumpolar Current (ACC), which helps to isolate Antarctica from the rest of the globe, maintaining its low and relatively stable temperatures (Rintoul *et al.* 2001). Close to the Antarctic continent, the average ocean temperature is approximately -1.8°C, with an annual fluctuation of less than 4°C (Barnes *et al.* 2006). In contrast to this remarkable stability, many features of the Southern Ocean display strong seasonality. One of the most striking features is the annual cycle of sea-ice growth and melt: ice coverage peaks to almost 20 million km^2 during the winter months, shrinking in summer to approximately 4 million km^2 (Parkinson *et al.* 1992). Fluctuations in sea-ice extent and sunlight exposure drive strong seasonal patterns of primary productivity, with phytoplankton growth in the Southern Ocean restricted to the late spring and summer months (summarised in Heywood & Whitaker 1984). This annual bloom of phytoplankton has a profound influence on the entire Southern Ocean ecosystem (Clarke 1988); both directly as a food source for zooplankton, and via subsequent trophic fluxes, including the settlement of organic matter to the seabed where a rich benthic community thrives.

1.1.2. Benthic habitats and fauna

In addition to strong seasonality and cold, stable temperatures, the benthic environment of the Southern Ocean is unique for several reasons (see Arntz *et al.* 1994 for a review). The continental shelf surrounding Antarctica is on average deeper than any other (Clarke & Johnston 2003). Ice exerts a particularly strong influence: scouring of the seafloor occurs via icebergs, glaciers, sea-ice and anchor ice, leaving patches of

benthic habitat completely devoid of fauna (reviewed in Gutt 2001). Ice disturbance, along with varied substratum types, light exposure and localised currents create a particularly heterogeneous benthic environment in Antarctica, providing a wide range of ecological niches (Gallardo 1987; Raguá-Gil *et al.* 2004; Barnes & Conlan 2007; Gutt 2007). This has led to a unique, rich and surprisingly diverse benthic fauna.

A great number of studies have assessed the overall biodiversity and biomass of the Antarctic benthos, and comprehensive reviews can be found in Clarke & Johnston (2003) and Arntz *et al.* (1997). Of note is the distinct absence of certain taxa such as the decapod crustaceans, whilst other groups – such as pycnogonids and peracarid crustaceans – appear to have radiated remarkably (see Watling & Thurston 1989; Brandt 2000; Clarke & Johnston 2003). Despite the heterogeneous nature of the environment, the Antarctic benthic fauna has long been considered a relatively homogenous unit (e.g. Hedgpeth 1970; Dayton 1990). This has been driven by assumptions that strong circumpolar currents promote the distribution of juveniles, ecological conditions are highly similar around the coast, and most species have a circumpolar distribution (see Gutt 2007). Even more recent attempts to classify the benthos have maintained relatively coarse biogeographical divisions (Clarke 2008).

Despite the considerable body of research on Antarctic benthic community patterns, there remains a dearth of comprehensive ecological, physiological and genetic studies on individual species (Griffiths 2010). Recent studies suggest that species distributions and biodiversity estimates must be revised, and that populations are much less homogenous than previously thought (Rogers 2007). The urgency to address these issues lies in the predicted vulnerability of Antarctic benthos to future environmental change. Many Antarctic benthic species are stenothermic (i.e. having a narrow range of thermal tolerance), have typically long generation times that may delay adaptation to change, and the isolation of the Antarctic coastline is likely to hinder migration to more favourable habitat (Clarke & Crame 1992; Peck 2005). Some regions of Antarctica are already experiencing rapid climate change (Vaughan *et al.* 2003; Meredith & King 2005), and the benthos is further threatened by destructive fishing practices, and potentially by localised pollution (Lenihan 1992; Clarke & Harris 2003; Stark *et al.* 2004).

1.1.3. Management initiatives

The Madrid Protocol (ratified by 27 countries, in force from 1998) recognises the importance of preserving Antarctic wildlife, and advocates the use of specifically protected areas to do so (Antarctic Treaty, 1991). The designation of protected areas has been focused largely on terrestrial ecosystems to date, and despite the many threats currently facing the Southern Ocean, the establishment of Marine Protected Areas (MPAs) in Antarctica lags well behind the rest of the world (Grant 2005; Antarctic and Southern Ocean Coalition 2008). In response, the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) is currently leading an initiative to establish a comprehensive network of MPAs in the Southern Ocean (CCAMLR 2005), with preservation of benthic ecosystems an important consideration. MPAs are one of the most powerful tools used to conserve marine life, yet their success relies strongly on a spatial design that ensures ecological robustness by considering the distribution, diversity, abundance and connectivity of associated fauna (Agardy 1994; Lubchenco *et al.* 2003; Shanks *et al.* 2003). Consequently, the establishment of MPAs in the Southern Ocean has been hindered by a lack of existing baseline knowledge on Antarctic marine fauna (Harris *et al.* 2007). The need to improve our understanding of the biodiversity and spatial heterogeneity of the Southern Ocean has recently been identified by the Australian Government as a key research priority, in order to build a stronger scientific foundation for spatial management of this fragile ecosystem (Australian Antarctic Division 2011).

1.2. GENETIC STRUCTURE

1.2.1. Why study genetic variation in marine populations?

The power of genetics to study marine populations is undisputed, yet marine genetic research significantly trails behind terrestrial work (Féral 2002). Examining intraspecific genetic variation provides an estimate of gene flow and consequently an overall picture of the dispersal occurring among populations ('connectivity') (Bohonak 1999; Hellberg *et al.* 2002). This is particularly important in marine environments because direct observations of dispersal are rarely logistically feasible (Palumbi 1995).

Genetic connectivity has received considerable attention in temperate and tropical marine systems, due to its role in informing spatial management: connectivity determines the degree of recruitment within protected areas and replenishment of populations outside a protected area (Botsford *et al.* 2001; Palumbi 2003; Sale & Kritzer 2003; Shanks *et al.* 2003). Studies have generally concluded that genetic connectivity in benthic species is determined by the duration of their pelagic larval phase (and therefore dispersal opportunity), however, exceptions abound and it is now acknowledged that many other factors can influence marine genetic structure (reviewed in Palumbi 1995).

Monitoring genetic structure and diversity in marine populations can help reveal the impact of anthropogenic contaminants ('genotoxicology'; see Hose 1994). This is a relatively new field (Schwartz *et al.* 2007), yet one of particular importance given that pollution currently poses one of the strongest threats to marine life (Costello *et al.* 2010). On a more general note, the study of genetic variation to identify local adaptation – be it to anthropogenic or natural selection pressures – can shed light on important speciation mechanisms (see Avise 2004). In the ocean, species boundaries may be associated more closely with chemical rather than morphological recognition, hence marine genetic studies have increasingly revealed a wealth of 'cryptic species' (Knowlton 1993, 2000); genetically-determined species that have previously remained hidden to traditional morphological taxonomy. Consequently, molecular tools now play a crucial role in marine biodiversity estimates (Bucklin *et al.* 2010). Another key purpose of genetic studies is to reveal the overall level of genetic diversity within populations, which determines their evolutionary potential in the face of environmental change (Lande & Shannon 1996; Hogg *et al.* 1998) and thus should be a conservation priority in order to ensure species' long-term persistence (Bowen 1999).

1.2.2. Genetic structure in Antarctic benthos

Research on the genetic structure and diversity of Antarctic benthic populations has been embraced only relatively recently (Griffiths 2010) and there remains a distinct lack of information from some regions (e.g. East Antarctica; see Grant & Linse 2009), and over smaller (i.e. population-level) scales (Held & Leese 2007). One of the most prominent findings to emerge from recent molecular work on Antarctic benthos has been a wealth of distinct genetic lineages, indicating cryptic species (e.g. Held &

Wägele 2005; Linse *et al.* 2007; Hunter & Halanych 2008; Wilson *et al.* 2009; Krabbe *et al.* 2010). These high levels of cryptic speciation imply that we may be drastically underestimating Antarctic biodiversity (Grant & Linse 2009), and that assumed 'circumpolar' distributions may in fact represent complexes of morphologically indistinguishable, yet genetically distinct sister species (Raupach *et al.* 2007; Rogers 2007; Lörz *et al.* 2009; Brandão *et al.* 2010).

A recent review found that approximately 90% of all genetic studies on Antarctic marine fauna examined either mitochondrial DNA or conserved nuclear DNA (18S and 28S) sequences (Grant & Linse 2009). It is no surprise then that intraspecific genetic variation remains poorly understood; indeed, the need to use faster evolving markers to explore population-level processes in Antarctic benthic fauna has been recognised (Held & Leese 2007; Hoffman *et al.* 2010). There is recent genetic evidence to suggest that Antarctic benthic populations may be isolated on relatively small scales, however, this is based on just a handful of studies (Allcock *et al.* 1997; Guidetti *et al.* 2006; Arango *et al.* 2010; Demarchi *et al.* 2010). Clearly this is an area that warrants further research, particularly given the need for improved connectivity estimates to help inform the design of Southern Ocean MPAs. Additionally, Antarctica provides an ideal setting for genotoxicological studies, as pollution is often highly localised (Lenihan *et al.* 1990; Kennicutt II *et al.* 1992; Snape *et al.* 2001). However this field has barely been addressed in Antarctic benthos; nor has localised adaptation in general.

1.3. ECOLOGICAL DYNAMICS

1.3.1. Benthic population ecology: global status

The life history traits and population dynamics of species (collectively referred to as 'population ecology' hereafter) reflect their adaptation to the environment (see Stearns 1976). Therefore knowledge of these parameters is crucial to predict the response of organisms and their communities to environmental change (e.g. Svensson *et al.* 2005; Ozgul *et al.* 2010). Much work has focused on the classification of life history strategies as either 'r-selected' or 'K-selected' (see Pianka 1970), although it is acknowledged that most organisms will sit somewhere on a continuum between the two. In marine benthos, there has been great emphasis on latitudinal trends – in

particular, the increase in body size and delayed onset of maturity with increasing latitude (e.g. Emson *et al.* 1989; Highsmith & Coyle 1991; Cardoso & Defeo 2004). Water temperature is widely accepted as the key driving factor of this trend, although the exact mechanisms that underlie this pattern are still debated widely (reviewed in Angilletta *et al.* 2004). Even more contentious is "Thorson's paradigm" - a proposed transition in reproductive strategy from broadcast spawning of larvae in the tropics to brooded, direct development at higher latitudes (Thorson 1950). More recent work suggests that the trend may be both taxa- and habitat-specific (Pearse 1994; Gallardo & Penchaszadeh 2001). With respect to population dynamics (e.g. patterns in abundance, population composition), marine benthic species vary over a broad range of spatial and temporal scales, reflecting a myriad of influential factors (see Kaiser *et al.* 2005).

Whilst there is a great body of work on larger-scale benthic patterns, it is important to further our understanding of population ecology through 'autecological' studies of individual species, as they may not always conform to accepted paradigms (Tenore & Coull 1980; Gray & Elliott 2009). Further still, ecological parameters such as growth rates and reproductive traits may differ *within* species, reflecting the adaptation of populations to local environmental conditions (e.g. Strong 1972). Monitoring species-specific population dynamics will help reveal the impacts of invasive species (reviewed in Parker *et al.* 1999), local anthropogenic disturbance (e.g. Tsutsumi 1987; Cardoso *et al.* 2005), and even global climate change (summarised in Parmesan & Yohe 2003).

1.3.2. Population ecology of Antarctic benthic fauna

Several ecological traits generally characterise Antarctic benthic fauna; many reflecting the latitudinal trends already mentioned. These include slow growth rates, delayed maturity, long embryological development, large adult body size (and uniquely, 'gigantism': Chapelle & Peck 1999), low fecundity and long life spans (compared to temperate and tropical organisms: see Arnaud 1977; White 1984; Brey & Clarke 1993; Arntz *et al.* 1994). It has thus been claimed that Antarctic fauna possess typically k-selected life histories (Clarke 1979; Gallardo 1987), although 'A-selection' – representing adaptation to adverse yet predictable conditions – has been raised as a more appropriate classification (Greenslade 1983). Growth and reproduction is often highly seasonal, coinciding with the annual phytoplankton bloom – although this does

not hold true for all taxa, and depends largely on the trophic niche occupied (see Clarke 1988; Pearse *et al.* 1991). Depth zonation has been widely observed (usually attributed to ice scouring, e.g. Dayton 1970; Gambi *et al.* 1994; Barnes 1999) and substrate type is often claimed to strongly shape benthic population composition (e.g. Everitt *et al.* 1980; Kirkwood & Burton 1988; Barnes 1995). Unfortunately, existing Antarctic autecological studies often lack sufficient spatial or temporal coverage (particularly over the winter months) largely due to the relative inaccessibility of Antarctica (Arntz *et al.* 1992; Urban & Mercuri 1998; Griffiths 2010). A lack of basic knowledge on the population ecology of benthic species has hampered the monitoring of anthropogenic impacts in Antarctica (Lenihan 1992; Stark *et al.* 2003a). There is also very little empirical research on the relationships between actual environmental variables and species abundances (Gutt 2007). Such relationships play an integral role in determining bioregions, which in turn provide a useful framework to assess appropriate areas for marine protection (Heap *et al.* 2005). Therefore, research in this field will be pertinent to address future spatial management goals in the Antarctic.

1.4. MODEL ORGANISMS: AMPHIPODS

1.4.1. Overall importance

Amphipods are an abundant, widespread, and remarkably diverse order of the peracarid crustaceans (see Barnard 1981 for diagnostic traits). They occupy almost every aquatic niche on the globe, forming an important part of many ecosystems (Bousfield 1978). Amphipods are divided into several suborders, the largest of which is the Gammaridea, which mostly contains benthic species. Like all peracarid crustaceans, amphipods brood their eggs in a pouch, or 'marsupium', from which offspring are released fully developed (Johnson *et al.* 2001). Hence there is no larval phase so dispersal is assumed to be restricted to the mobility of adult forms, or 'rafting' on floating substrata (see Highsmith 1985). There is speculation that this brooding life history contributes to the high species diversity of amphipods by promoting the isolation of populations (Crisp 1978; Cohen & Johnston 1987; Mashiko 2000) - making studies of genetic connectivity in this group particularly interesting. With estimates of over 100 new species described each year (De Broyer & Jażdżewski 1996), and further

taxonomic incongruences revealed recently by molecular work (Havermans *et al.* 2010), basic taxonomy in the Amphipoda requires further examination. This is particularly important because amphipods are ideal subjects for environmental monitoring due to relatively small body size, high abundance, and reported environmental sensitivity (Thomas 1993). Indeed, they are commonly used as indicator species (see Conlan 1994).

1.4.2. Significance to Antarctic benthic studies

Amphipods are one of the most speciose macrobenthic groups in Antarctica, where they frequently dominate benthic communities (Jażdżewski *et al.* 1991; Stark 2000; De Broyer *et al.* 2003b; Brokeland *et al.* 2007). They play a crucial role in the Antarctic marine ecosystem and have at least 190 predatory species including fish, penguins, seals and invertebrates (Dauby *et al.* 2003). In fact, an estimated 60 million tonnes of amphipods are consumed in the Southern Ocean each year, making them the second most important prey item after krill (Dauby *et al.* 2003). Amphipods can reach very high densities in Antarctica: epifaunal gammaridean amphipods have been observed at densities up to ~300,000 individuals/m^2 (Amsler *et al.* 2008), and individual species estimates have been recorded as high as 17,000 individuals/m^2 (Jażdżewski *et al.* 1991). These astounding abundances, as well as their survival in a wide spectrum of ecological niches and high levels of species endemicity (De Broyer *et al.* 2007) reflect the success of amphipods in Antarctica and they appear to have undergone explosive radiation in this environment (Knox & Lowry 1977; Brandt 1999). This makes amphipods particularly appropriate candidates to investigate biodiversity and speciation processes in Antarctica (Watling & Thurston 1989; De Broyer *et al.* 2003b).

The general life history strategies of Antarctic amphipods – where known – often exemplify life adapted to the cold (Brandt 1999; De Broyer *et al.* 2003b), so they have also been identified as ideal models in which to compare the ecological consequences of life at different latitudes (Highsmith & Coyle 1991). However, there is still much information lacking on their basic population dynamics (Brandt 2000), making such comparisons limited. A review by Arntz *et al.* (1992) found that of the 600 Antarctic gammaridean amphipod species recorded at the time, detailed growth and reproductive data was available for just five of them. Improving our understanding of

ecologically-dominant taxa such as the Amphipoda will be critical to help predict the response of Antarctic ecosystems to environmental change (Harley *et al.* 2006).

1.5. THESIS OBJECTIVES AND OUTLINE

The overall aim of this thesis was to improve our understanding of the ecological processes structuring Antarctic benthic populations in order to better predict the effects of environmental change, and ultimately help inform decisions concerning the preservation of the unique Antarctic benthic ecosystem. These objectives were addressed using amphipods as a model organism. Study species were selected to represent dominant, ubiquitous members from each of two contrasting habitats: shallow coastal waters, and deeper continental shelf waters.

From the continental shelf, the sibling species *Eusirus perdentatus* (Figure 1.1) and *Eusirus giganteus* were used to investigate moderate-scale (100km) and large-scale (circum-Antarctic) connectivity, as well as the presence of cryptic genetic lineages - considered probable given the previous taxonomic instability of these species and the general lack of information on Antarctic biodiversity (Chapter 2). DNA sequencing was considered the most appropriate tool over these scales, and both mitochondrial and nuclear sequences were analysed, so that the unique processes affecting each part of the genome did not ultimately bias our view of genetic structure.

The fragmented nature of the Antarctic shallow coastal environment is expected to promote smaller-scale population structure, hence, genetic connectivity and diversity were explored in the nearshore species *Orchomenella franklini* (Figure 1.2) over distances as small as 100m, and up to 1000km (Chapter 3). Highly variable microsatellites were employed for this study as they provide finer-scale resolution than DNA sequences; also there is almost no information on microsatellite variability for any nearshore Antarctic benthic species.

To understand how the ecology of *O. franklini* is related to the nearshore Antarctic environment in which it thrives, population dynamics, abundance and distribution were deduced from ecological measurements (Chapter 4). Samples were analysed from a range of locations and times so that preliminary insights into spatial and temporal fluctuations in population ecology could be made. The association

between benthic sediment properties and amphipod distribution was also examined: a relationship that is particularly poorly understood in Antarctica.

These studies are drawn together in the General Discussion (Chapter 5) to shed light on the biodiversity, genetic structure and population ecology of Antarctic benthic amphipods as a predictor of other benthic brooding invertebrates. Implications for the vulnerability of these organisms to environmental change are discussed, as well as priorities for spatial management and future research in this isolated and unique area of the world.

1.5.1. Note on thesis structure

All data chapters within this thesis have been prepared as independent, self-contained manuscripts for publication. Chapter 2 has already been published in a peer-reviewed journal and Chapter 3 is currently under peer review – see individual chapters for details. Chapter 4 is yet to be submitted to a peer-reviewed journal.

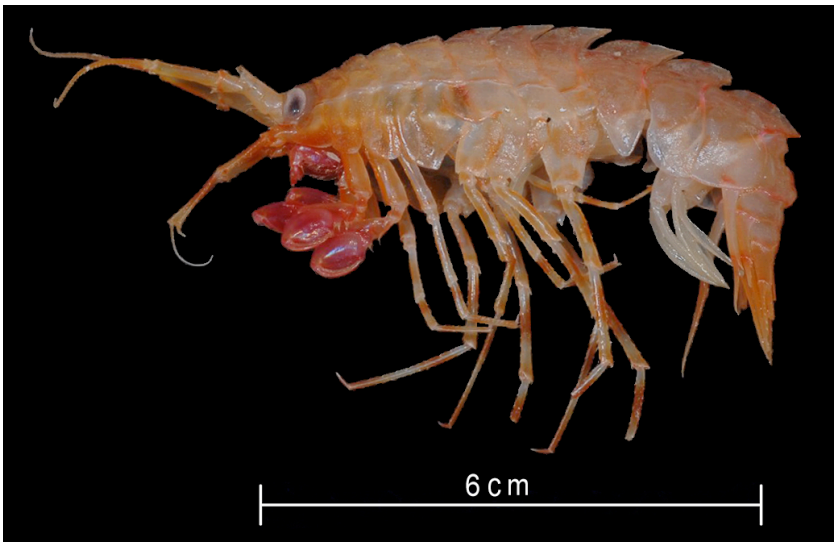

Figure 1.1: Adult specimen of the morphospecies *Eusirus perdentatus* (photo: H. P. Baird).

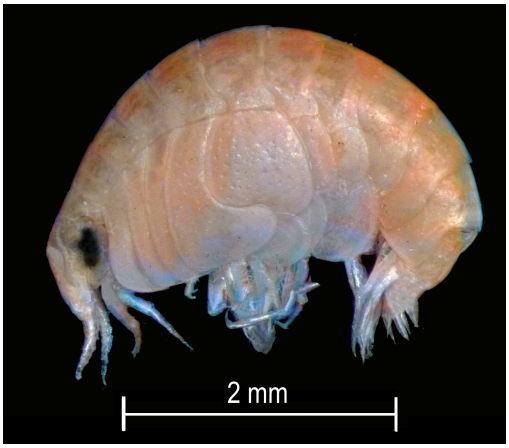

Figure 1.2: Adult specimen of *Orchomenella franklini* (photo: H. P. Baird).

Chapter 2. Evidence of hidden biodiversity, ongoing speciation and diverse patterns of genetic structure in giant Antarctic amphipods

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2.1. ABSTRACT

Recent molecular research on Antarctic benthic organisms has challenged traditional taxonomic classifications, suggesting that our current perceptions of Antarctic biodiversity and species distributions must be thoroughly revised. Furthermore, genetic differentiation at the intraspecific level remains poorly understood, particularly in eastern Antarctica. We addressed these issues using DNA sequence data for two sibling amphipod species that could be collected on a circum-Antarctic scale: *Eusirus perdentatus* and *Eusirus giganteus*. Haplotype networks and Bayesian phylogenies based on mitochondrial (COI, CytB) and nuclear (ITS2) DNA provided strong evidence of multiple cryptic species of *Eusirus*, with several occurring in sympatry and at least one likely to have a true circum-Antarctic distribution. Within species, gene flow was often highly restricted, consistent with a brooding life history and in some cases suggestive of current or future allopatric speciation. Patterns of genetic structure were not always predictable: one cryptic species showed preliminary evidence of high genetic differentiation across ~150km in eastern Antarctica (F_{ST} > 0.47, $p < 0.01$), yet another was remarkably homogenous across ~5000km ($F_{ST} = 0.00$, $p = 1.00$). Genetic diversity also varied among cryptic species, independent of sample size ($\pi = 0.00$-0.99). These results indicate several hidden levels of genetic complexity in these Antarctic amphipods that are neither apparent from previous taxonomic or ecological studies, nor predictable from their life history. Such genetic diversity and structure may reflect different modes of survival for Antarctic benthic organisms during historic glacial cycles, and/or subsequent re-establishment of populations on the shelf, and highlights our misunderstanding of Antarctic marine species diversity.

2.2. INTRODUCTION

Our oceans harbour overwhelming biodiversity, the vast majority of which remains to be discovered. Approximately 230,000 marine species are currently known, yet well over one million are estimated to exist (Bouchet 2006; Bucklin *et al.* 2010; Costello *et al.* 2010). Unfortunately, many of these are likely to disappear before we can describe them: marine biodiversity is under unprecedented threat from numerous anthropogenic factors, including climate change, pollution, introduced species, habitat destruction and overfishing (reviewed in Costello *et al.* 2010). In order to monitor and predict future changes to marine life, and ultimately to conserve it, there is an urgent need to better understand and document the distribution and abundance of marine species.

In the quest to understand marine biodiversity, research at the genetic level has gained popularity as it sheds light on the fundamental evolutionary processes that ultimately drive species diversity (Féral 2002; Goetze 2003). Exploring genetic variation also improves our ability to document biodiversity, by aiding species discrimination through techniques such as DNA barcoding (Blaxter & Floyd 2003; Hebert *et al.* 2003a; Bucklin *et al.* 2010). This is pertinent in the marine realm, where there is a prevalence of morphologically cryptic species which can remain undetected by traditional taxonomic methods (Knowlton 1993). In terms of the ongoing protection of biodiversity, intraspecific genetic variation and structure provide crucial information regarding the potential of an organism to adapt and speciate (Erwin 1991; Lande & Shannon 1996). Consequently, the spatial design of many marine reserves is now largely based on estimates of the genetic structure within species (Féral 2002; Palumbi 2003; DeSalle & Amato 2004).

One particularly vulnerable marine ecosystem that harbours surprisingly high diversity is the Antarctic benthos. Antarctic benthic organisms – many of which are endemic – have poor prospects for surviving climate change (Clarke & Crame 1992; Peck 2005), yet Antarctica is currently experiencing some of the most rapid climate change on earth (Meredith & King 2005). Molecular studies have only been embraced relatively recently to investigate Antarctic biodiversity (Grant & Linse 2009; Griffiths 2010) and these studies have revealed an astounding prevalence of cryptic speciation within the benthos (summarised in Janosik & Halanych 2010). The discovery of hidden diversity not only raises doubt over current estimates of Antarctic biodiversity, but also

suggests that geographic distributions of Antarctic benthic fauna must be revised. Several taxa previously assumed to be circum-Antarctic are now known to be a complex of geographically restricted cryptic species (Wilson *et al.* 2007; Lörz *et al.* 2009; Brandão *et al.* 2010). Even when species do maintain a wide Antarctic distribution, populations are likely to be genetically isolated (Rogers 2007). Whilst genetic connectivity across oceanographic barriers such as the Polar Front has been addressed in several benthic species, true circum-Antarctic genetic structure (i.e. restricted to the continental shelf) remains poorly understood (Held & Leese 2007; Hoffman *et al.* 2010). Resolution has been hampered by the limited geographic spread of most Antarctic molecular studies and a particular dearth of samples from the east Antarctic (Clarke *et al.* 2007a; Grant & Linse 2009). However, a recent study of the pycnogonid *Nymphon* provided evidence of genetic subdivision between eastern Antarctic populations separated by less than 30km (Arango *et al.* 2010).

Peracarid crustaceans – in particular amphipods and isopods – have been flagged as ideal candidates to explore genetic patterns in the Antarctic (Watling & Thurston 1989; De Broyer *et al.* 2003b; Rogers 2007). They are highly abundant and diverse on the continental shelf and often exemplify characteristics of Antarctic benthic fauna such as eurybathy, gigantism and endemicity (De Broyer 1977; White 1984; Brey *et al.* 1996; Brandt 1999). Possessing strictly brood development and therefore a limited dispersal capacity, intraspecific genetic variation is likely to be highly structured in peracarids (De Broyer *et al.* 2003b). Whilst Antarctic isopods have been subject to numerous intraspecific molecular investigations (Held 2003; Held & Wägele 2005; Raupach & Wägele 2006) – all of which have detected evidence of cryptic species – Antarctic amphipods are less studied in this respect. This is surprising given their frequent use as indicator species (Lahdes *et al.* 1993; Duquesne *et al.* 2000; Young *et al.* 2006) and their crucial role as a trophic resource, with at least 190 species of predator in the Southern Ocean (Dauby *et al.* 2003). Furthermore, the morphological taxonomy of amphipods is known to be especially controversial, warranting molecular attention (Radulovici *et al.* 2009; Havermans *et al.* 2010).

The large size and relative abundance of the Antarctic amphipod *Eusirus perdentatus* (Chevreux 1912) have made it a popular subject for ecological studies (Klages & Gutt 1990; Klages 1993; Dauby *et al.* 2001; De Broyer *et al.* 2001). However, a recent re-evaluation of the morphology of *E. perdentatus* revealed a second species: *Eusirus giganteus* (Andres *et al.* 2002). Only narrow morphological

distinctions separate the two, and it is likely that some previous identifications of *E. perdentatus* have in fact represented *E. giganteus* specimens. Therefore, the behaviour, diet, and life history strategies determined for *E. perdentatus* in previous studies may be erroneous. The reportedly wide bathymetric and geographic distributions of these sister species – despite a brooding mode of reproduction – suggested that they comprised genetically isolated populations, or potentially even more hidden species. We explored genetic diversity in *E. perdentatus* and *E. giganteus* using DNA sequence data, with an aim to: a) confirm their taxonomic status and test for evidence of additional cryptic species within the genus; and b) explore intraspecific genetic structure on a circum-Antarctic scale.

2.3. METHODS

2.3.1. Sample collection

Eusirus amphipods were collected from five locations around Antarctica (Figure 2.1). These represent currently proposed bioregions based on Antarctic benthos (Clarke *et al.* 2007a), and are each separated by ≥ 1500km. Within each location, animals were collected from up to 5 sites with an average of 273km separation among sites (Table S2.1[1]). Collections were made by benthic trawl or dredge from depths of 295-930m (Table S2.1). All samples were collected during the austral summer, over the years 2002–2010. *Eusirus* specimens were preserved in 80-100% ethanol as soon as possible after collection and were identified as either *E. perdentatus* or *E. giganteus* based on morphological characters, according to the diagnostic key provided in Andres *et al.* (2002).

[1] All supplementary figures and tables for this chapter are provided in Appendix I.

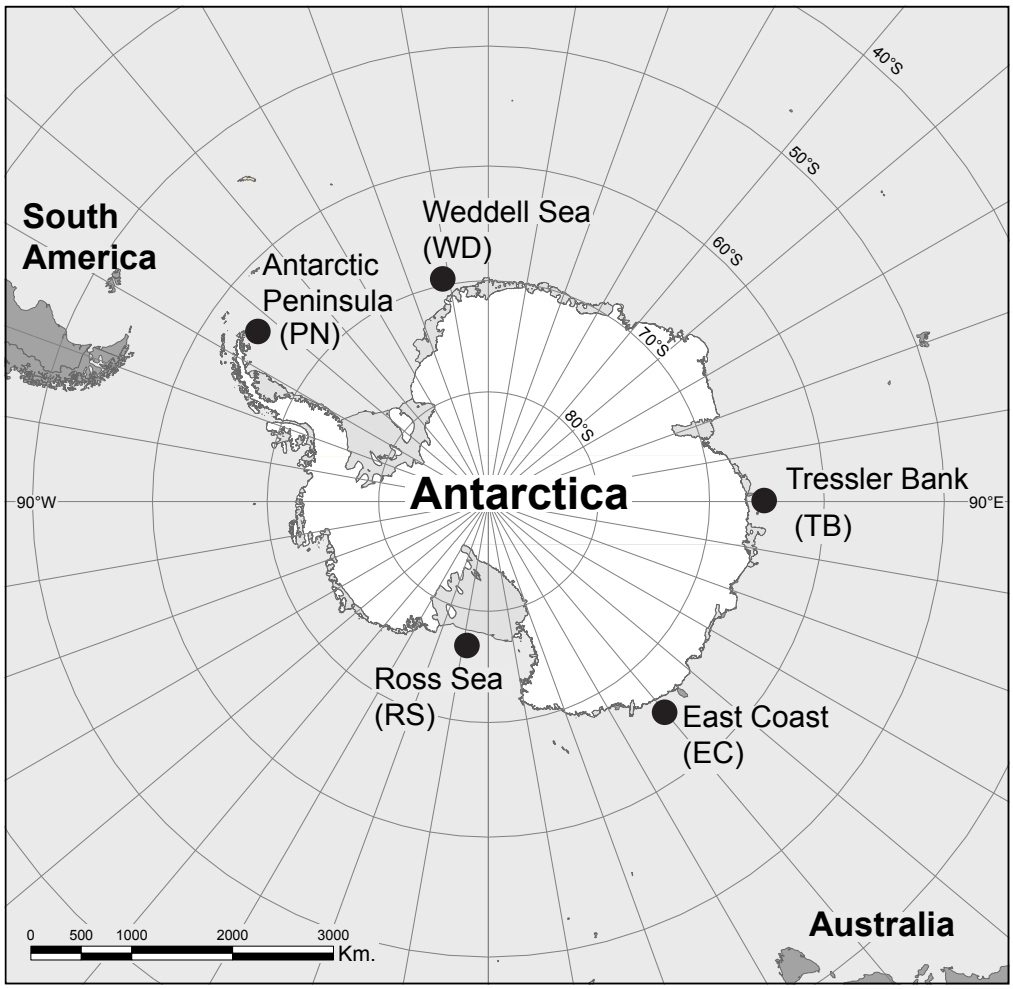

Figure 2.1: Map of Antarctica showing the five sample collection locations - the Antarctic Peninsula (PN), Weddell Sea (WD), Tressler Bank region (TB), East Coast (EC), and Ross Sea (RS). The numbers of sites sampled within each location are: PN = 4, WD = 4, TB = 4, EC = 5, RS = 5 (see Table S2.1 for site details). Based on Map 13351 courtesy of the Australian Antarctic Division.

2.3.2. DNA extraction, amplification and sequencing

Genomic DNA was extracted from the first left pleopod of each animal (for consistency and to minimise contamination from parasites or gut content) using the DNeasy Blood and Tissue Kit (QIAGEN). Protocols were carried out according to the manufacturer's instructions but with elution volume decreased to 120µl to maximise DNA concentration. Final concentrations of genomic DNA were determined using a NanoDrop 8000.

We amplified three DNA regions in order to compare sequence variation among geographic locations because several independent assessments of variation – particularly from nuclear and mitochondrial DNA – allow a more robust inference of genetic structure than a single DNA region (Edwards & Beerli 2000). The internal transcribed spacer region 2 (ITS2) is a nuclear DNA region that has shown considerable variation in arthropods at the species level or lower (e.g. Hackett *et al.* 2000; Oh *et al.* 2009; Yao *et al.* 2010). Similarly, the mitochondrial genes cytochrome c oxidase subunit 1 (COI) and cytochrome b (CytB) are highly polymorphic DNA regions that have been useful to elucidate both inter- and intra-specific relationships in crustacean taxa (e.g. Bucklin *et al.* 1997; Schizas *et al.* 1999; Carlini *et al.* 2009).

Polymerase chain reactions (PCRs) were in a final volume of 50μl, comprising 10-300ng DNA, 200nM each primer (Table 2.1), 200μM each dNTP, 2mM MgCl, 1x PCR Buffer (Bioline), 0.5x BSA and 1 Unit BioTaq Red DNA Polymerase. Reactions were carried out on a Bio-Rad DNA Engine Tetrad Thermal Cycler, and always included a negative control. Individual thermal cycling parameters for each primer set are provided in Table 1, and all protocols began with 2 minutes at 94°C and ended with 5 minutes at 72°C. PCR products were purified using a QIAGEN PCR Purification Kit and quantified on the NanoDrop 8000. Bi-directional sequencing was carried out at the Australian Genome Research Facility (Brisbane) on an AB 3730xl sequencer. Sequences were assembled in MEGA 4 (Tamura *et al.* 2007), aligned with ClustalW default settings, and further improved with manual modifications. Intragenomic variation, which is notorious in crustacean ITS sequences (Chu *et al.* 2001), was observed in only a few individuals, so we omitted these individuals from the analysis altogether. Whilst this conservative approach resulted in a smaller dataset for ITS2, it ensured that all sequence data was unambiguous, so that phylogenetic patterns would not be confounded by intragenomic variation. ITS2 sequences also contained several indels of considerable length (another phenomenon common to crustacean ITS sequences; e.g. Carlini *et al.* 2009; Brandão *et al.* 2010). Indels were retained due to strong evidence that they contain useful phylogenetic information (Giribet & Wheeler 1999; Simmons *et al.* 2007). However we used GapCoder (Young & Healy 2003) to re-code indels as a single character representing the absence or presence of a single event (Vogler & DeSalle 1994; Carlini *et al.* 2009), as treating each gap as a fifth character would have weighted each indel event too severely.

Table 2.1: Primer sequences, corresponding references, and PCR cycles used to amplify cytochrome b (CytB), cytochrome oxidase I (COI) and internal transcribed spacer 2 (ITS2).

Locus	Primers (5' - 3')	Primer reference	Thermal cycling protocol
CytB	151F: TGTGGRGCNACYGTWATYACTAA 270R: AANAGGAARTAYCAYTCNGGYTG	Merritt *et al.* (1998)	[60s at 94°C, 60s at 51°C, 70s at 72°C] x 38
COI	LCO1: GGTCAACAAATCATAAAGATATTGG HCO2: TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.* (1994)	[30s at 94°C, 45s at 51°C, 60s at 72°C] x 34
ITS2	ITS4: TCCTCCGCTTATTGATATGC ITS5: GGAAGTAAAAGTCGTAACAAGG	White *et al.* (1990)	[30s at 94°C, 45s at 61°C, 60s at 72°C] x 30

2.3.3. Phylogenetic analyses

The relationship among haplotypes and their geographic distribution was explored with haplotype networks. TCS 1.21 (Clement *et al.* 2000) was used to create maximum parsimony networks for each DNA region, with 95% connection limits and geographic distribution of haplotypes overlayed on the networks. Phylogenetic relationships among individuals of the morphologically identified *E. perdentatus* and *E. giganteus* were examined using Bayesian analysis in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The three DNA regions were concatenated for analysis, yet each region within the concatenated sequence was treated under an individual model of substitution. The most appropriate model for each DNA region was determined using Modeltest 3.7 (Posada & Crandall 1998); these were TrN+G for CytB, K80+I+G for COI, and K81+I for ITS2. If the exact model was not an option in MrBayes, the next most complex model was used. Indels that had been re-coded as single events in the ITS2 sequence were treated as binary data, adjusting for the ascertainment bias that indels present or absent in all taxa cannot be observed. We ran two simultaneous sets of four MCMC chains (one cold, three hot) for $2x10^6$ generations, sampling trees every 100 generations. Log likelihood values were plotted against generation for each run to determine convergence; burn-in was accordingly set at a conservative level of 25% (5000 trees). A consensus tree with nodal posterior probability support was obtained from the remaining 15,000 sampled trees. The tree was unrooted as no suitable outgroup was available, due to the numerous and large indels within ITS2 that made alignment with all other available amphipod sequences highly ambiguous. MEGA 4 was used to determine sequence divergence (corrected p-distances) between and within the clades indicated by the phylogenetic tree, as well as between the two species defined originally by morphology. These distances were tested for conformation to the "4x" criterion (Birky *et al.* 2005), which states that clades are truly independent lineages worthy of species status when interclade divergence exceeds four times the intraclade divergence. Although initially developed for asexual organisms, the "4x" rule can also be applied to mitochondrial data, and has been used as a conservative approach to delimit cryptic species in crustaceans (Morrone *et al.* 2010).

2.3.4. Population structure and diversity

Phylogenetic analysis identified 7 distinct genetic clades within the *Eusirus* sampled (see Section 2.4.2.). We explored neutrality of nucleotide variation and population demographics for each of these genetic clades as Tajima's *D* and Fu's F_S statistics generated in Arlequin 3.11 (Excoffier *et al.* 2005), with significance tested by 10,000 permutations (a Bonferroni correction was also applied to account for multiple comparisons between clades, adjusting significance at the 0.05 level to $p < 0.0024$). Estimates of haplotype diversity (*h*) and nucleotide diversity (π) were also calculated for each clade. Due to marked differences between the diversity of clades, we explored whether π was correlated with sample size, using Pearson's correlation coefficient (r). To investigate whether further diversity remained hidden due to undersampling, we generated haplotype rarefaction curves, using EstimateS 8.2.0 (Colwell, 2009). Both individual-based curves and sample-based curves (for definitions see Gotelli & Colwell 2001) were generated by 500 randomisations of sampling order without replacement.

We estimated genetic differentiation among locations and sites as F_{ST} for each DNA region using Analysis of Molecular Variance (AMOVA). With the discovery of so many clades potentially indicating cryptic species, intraspecific sample size at each location and site was drastically reduced. Only four estimates could be made among locations, and one among sites. AMOVAs were performed using the distance matrix option in Arlequin 3.11, and statistical significance was evaluated with 10,000 permutations.

2.4. RESULTS

2.4.1. Overall sequence variation

Amplification success for all three DNA regions was high and sequence peaks were typically clean. A small proportion of sequences (~3% for CytB, ~10% for COI and ~19% for ITS2 - the latter also reflecting intragenomic variation) were suboptimal and therefore discarded, leading to the different dataset sizes for each locus. A total of 117 CytB sequences which were 376bp in length revealed 95 variable sites (89 parsimony-informative) and 36 unique haplotypes. Analysis of 109 COI sequences

620bp in length revealed 132 variable sites (129 parsimony-informative) and 45 haplotypes. Once ITS2 indels were re-coded as single absence/presence characters, sequence length was 457bp, 107 sites were variable (98 parsimony-informative) and 33 unique haplotypes were recovered from 98 individuals (length of the original ITS2 dataset including alignment gaps was 506bp. CytB and COI sequences contained no indels. All DNA regions had high levels of diversity (CytB: $h = 0.940$, $\pi = 0.071$; COI: $h = 0.960$, $\pi = 0.063$; ITS2: $h = 0.914$, $\pi = 0.053$).

2.4.2. Hidden diversity within *E. perdentatus* and *E. giganteus*

Our results indicate multiple distinct genetic groups exist within the two morphologically defined species *E. perdentatus* and *E. giganteus*. Both morphological species were separated into several unconnected haplotype networks based on all three DNA regions, indicating the presence of cryptic species (Hart & Sunday 2007). Mitochondrial regions each revealed the same eight distinct networks (Figure 2.2a,b), whilst ITS2 joined two of these (g4a and g4b) to produce seven separate networks (Figure 2.2c). Interestingly, when maximum parsimony connection limit was reduced to 90%, CytB also connected g4a and g4b, so we chose conservatively to define just the seven networks as distinct genetic groups (clades p1 - p3 within *E. perdentatus*, and g1 - g4 within *E. giganteus*, Figure 2.2). Bayesian phylogenetic analysis based on each DNA region produced clades concordant with haplotype networks, and confirmed the monophyly of each clade. Analysis of the concatenated sequence provided overwhelming support for the seven major monophyletic clades, with all posterior probabilities ≥ 0.99 (Figure 2.3).

The level of sequence divergence among the seven genetic clades is consistent with cryptic speciation. Mean inter-clade divergence for CytB ranged from 6.3% to 13.8%, for COI ranged from 4.6% to 10.9%, and for ITS2 ranged from 4.9% to 11%. These ranges reflected values typically observed between known congeneric species in a variety of other taxa (CytB: Kartavtsev & Lee 2006, COI: Hebert *et al.* 2003b, ITS2: Yao *et al.* 2010). Mean sequence divergence between clades was always higher than the divergence within clades, with no overlap of values (Figure 2.4); a key criteria used to delimit cryptic species (Hebert *et al.* 2003b; Meyer & Paulay 2005). Furthermore, all clades also satisfy the "4x" rule (Birky *et al.* 2005): overall mean interclade divergence was 15x interclade divergence for both mitochondrial regions, and in all pairwise

comparisons, interclade distances exceeded 4x intraclade distances. Overall COI divergence between the two morphological species (*E. perdentatus* and *E. giganteus*: 8.3% uncorrected divergence) was often less than the COI divergence between clades within each morphological species (*e.g.* 9.1% uncorrected divergence between clades g2 and g3). This discrepancy between morphological and genetic distinctions was the same for CytB and ITS2, strongly indicating that the clades indeed represent cryptic species. Finally, all COI divergence values between clades exceeded the COI species screening threshold (SST) developed for amphipods by Witt *et al.* (2006) (SST = 3.75% divergence; minimum divergence between *Eusirus* clades = 4.4%).

2.4.3. Genetic variation within clades

Nucleotide diversity varied between the seven clades (Table 2.2) and was not correlated to sample size ($p > 0.05$ for all DNA regions). Haplotype rarefaction curves provided strong evidence that more haplotypes would be revealed by increasing the number of sites sampled or the number of individuals sequenced, as neither sample-based curves nor individual-based curves for any of the DNA regions reached an asymptotic shape (see Figure S2.1 for more detail).

Neutrality for each of the DNA regions was generally upheld. Tajima's *D* was significantly high for COI and CytB within clade g4 (Table 2.2), however this most likely reflected the high genetic subdivision detected within this clade for these gene regions. Significant negative values of F_S for COI and ITS2 within the large clade p3 potentially indicated population expansion, although only ITS2 remained significant after Bonferroni correction (Table 2.2). In clade g1, a significantly negative F_S value was detected for CytB alone, possibly suggesting selection at this DNA region (Table 2.2). However the value did not remain significant after Bonferroni correction, and may have been influenced by the large degree of geographic structure in this clade (see Section 2.4.4.).

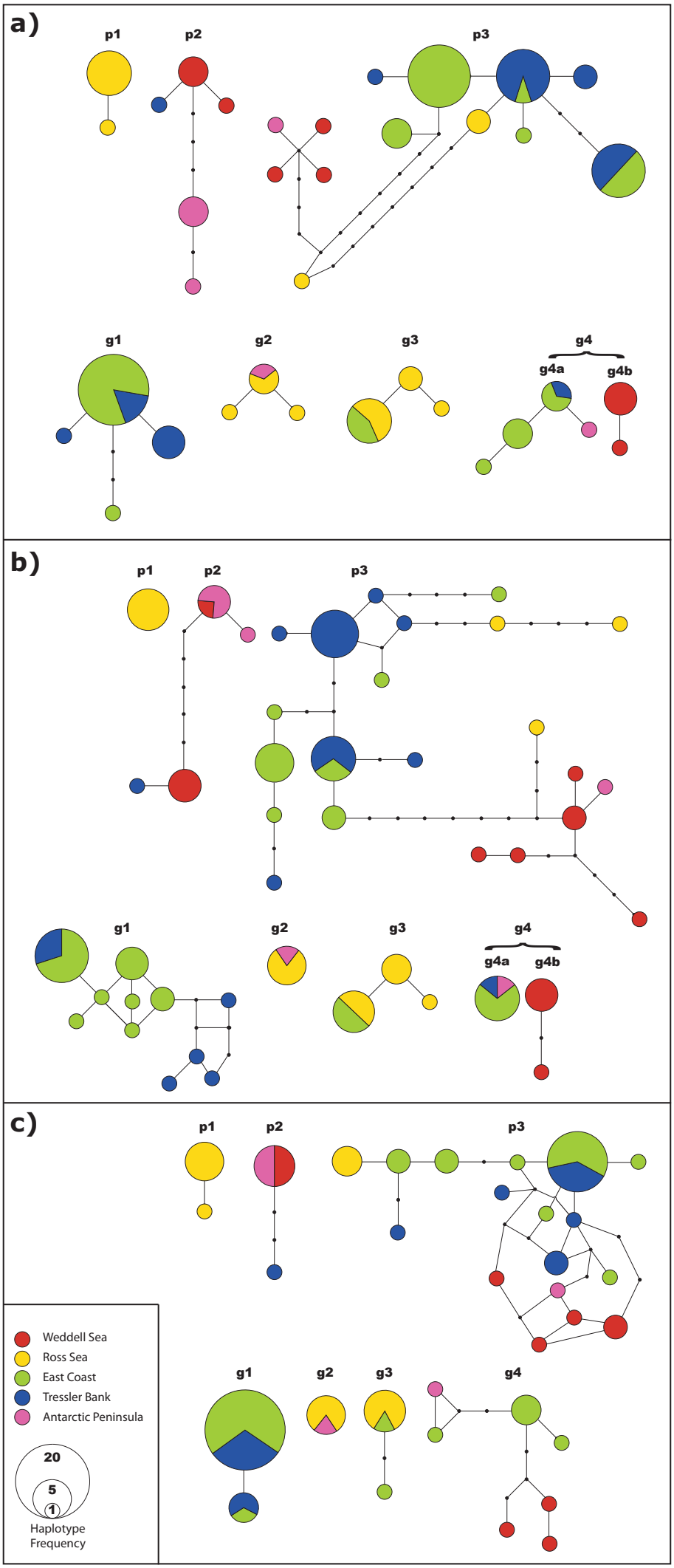

Figure 2.2: Statistical parsimony haplotype networks for cytochrome b (a), cytochrome oxidase subunit I (b) and internal transcribed spacer 2 (c). The area of each circle is proportional to the frequency of the haplotype, and nodes represent unsampled or extinct haplotypes. Colours represent the location from which corresponding samples were collected (see legend). Designated clades are labelled with the prefix 'p' or 'g', indicating their original taxonomic identification as either *E. perdentatus* or *E. giganteus*, respectively.

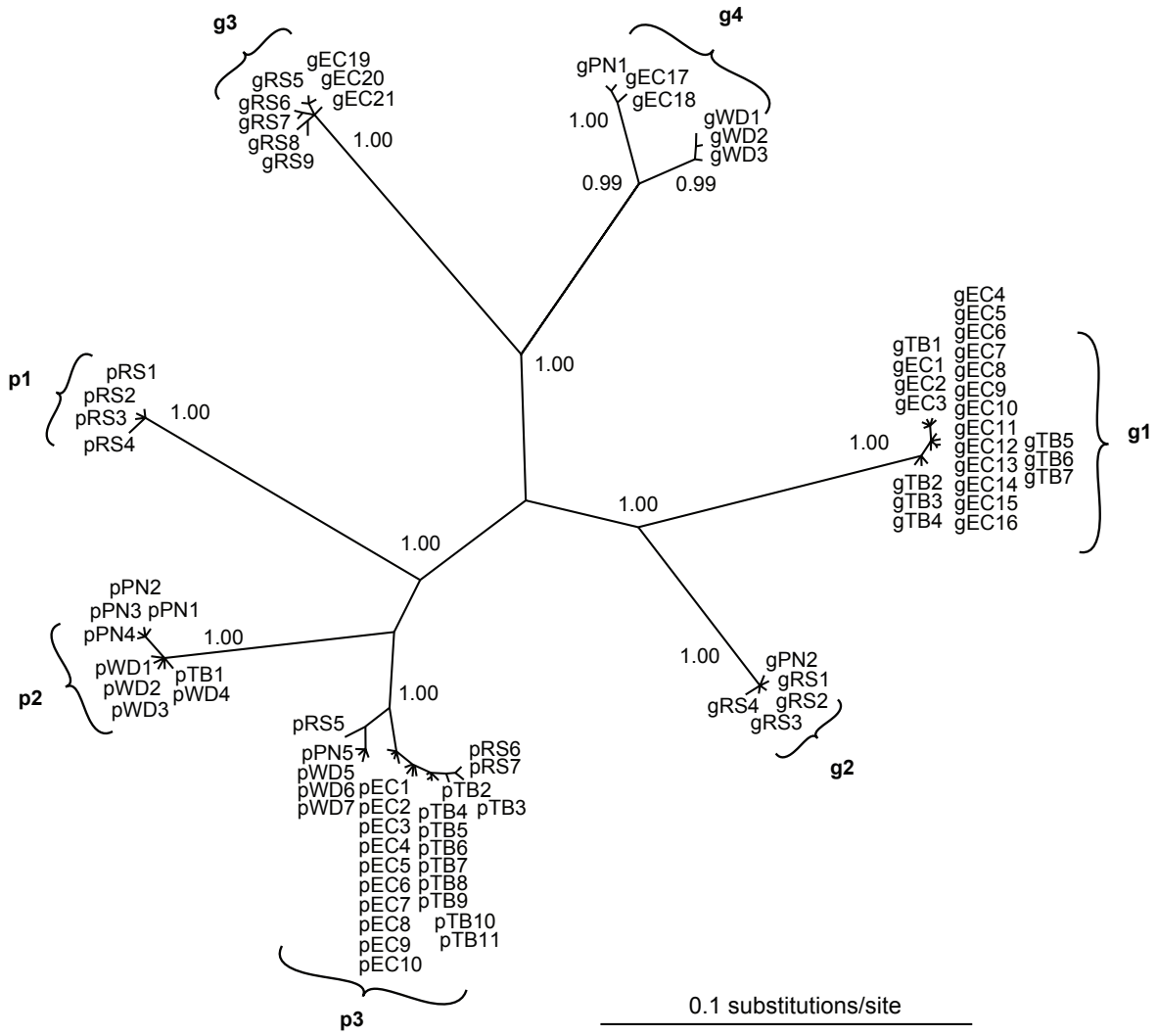

Figure 2.3: Unrooted Bayesian consensus tree of concatenated dataset (COI + CytB + ITS2). Values represent posterior probabilities. Individuals are denoted by sample locations, with a prefix indicating their original taxonomic identification (p: *E. perdentatus*; g: *E. giganteus*). The seven main clades as determined from haplotype networks are indicated.

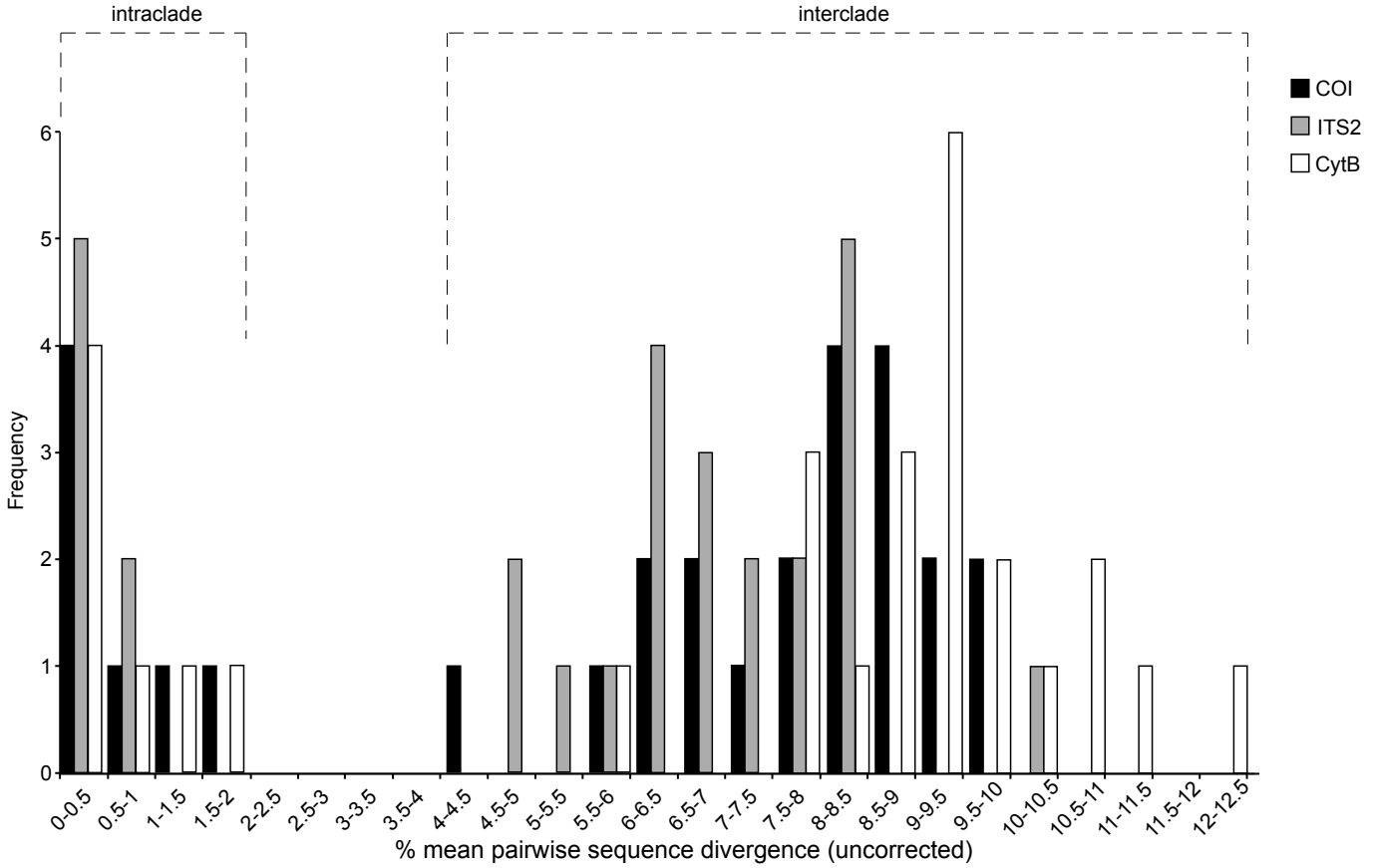

Figure 2.4: Histogram showing frequency of mean uncorrected sequence divergence within and between clades. Shading corresponds to DNA region (see legend).

Table 2.2: Genetic diversity and neutrality indices for each clade. n: sample size, *K*: number of haplotypes, *h*: haplotype diversity, π: nucleotide diversity. Significance of Tajima's *D* and Fu's F_S values represented with asterisk: *p < 0.05; **p < 0.01 (also **p < 0.05 after Bonferroni correction).

	Cytochrome b						Cytochrome oxidase subunit I						Internal transcribed spacer 2					
Clade	n	*K*	*h*	π	Tajima's *D*	Fu's *Fs*	n	*K*	*h*	π	Tajima's *D*	Fu's *Fs*	n	*K*	*h*	π	Tajima's *D*	Fu's *Fs*
p1	8	2	0.2500	0.0007	-0.1820	0.2007	6	1	0.0000	0.0000	-	-	6	2	0.3333	0.0007	-0.9330	-0.0028
p2	9	5	0.8333	0.0081	0.8314	0.0871	10	4	0.7333	0.0060	1.3814	2.1256	9	2	0.2222	0.0014	-1.5130	1.3176
p3	48	13	0.8351	0.0106	-0.5120	-1.1751	41	22	0.9402	0.0105	-0.3595	-5.8609*	35	17	0.8571	0.0064	0.0989	-8.4201**
g1	24	4	0.4239	0.0017	-1.5491*	-0.9025	25	11	0.9867	0.0044	0.1102	-2.3439	26	2	0.2123	0.0005	-0.3105	0.1618
g2	5	3	0.7000	0.0021	-0.9726	-0.8292	5	1	0.0000	0.0000	-	-	5	1	0.0000	0.0000	-	-
g3	10	3	0.5111	0.0023	-0.6575	0.2060	10	3	0.6000	0.0012	0.1203	-0.1006	8	2	0.4286	0.0019	0.4142	1.6533
g4	13	6	0.8462	0.0169	1.6992*	2.0049	12	3	0.5909	0.0165	2.3706**	9.4542**	9	7	0.9167	0.0064	0.0871	-2.5467
Total	*117*	*36*	*0.9397*	*0.0707*			*109*	*45*	*0.9602*	*0.0626*			*98*	*33*	*0.9142*	*0.0530*		

2.4.4. Geographic structure of clades

One clade (p1) within the *Eusirus* species complex was restricted entirely to a site within the Ross Sea, but all other clades occurred in two or more locations (Figure 2.2). Only clade p3 was truly "circum-Antarctic" in distribution, containing samples from all five locations (Figure 2.2). However, this may have been an artefact of limited replication within regions and we acknowledge that further sampling may well increase the known geographic range of other clades. Several clades occur in sympatry, not only occurring at the same location but also the same site. For example, individuals from clades p2, p3 and g1 were all found at site TBc. This site represents just a single trawl covering $2km^2$ over a 20m depth gradient, therefore these distinct clades exist in close geographic and bathymetric proximity.

Individual haplotypes were only occasionally shared between locations, and usually these locations were adjacent. Notably in clade g2, an identical haplotype was shared between the Antarctic Peninsula and Ross Sea, for all three DNA regions. There was also a single COI haplotype shared across over 8000km – occurring in the East Coast, Tressler Bank and Antarctic Peninsula locations.

Within clades there is evidence of geographic structure indicating limited gene flow and dispersal. AMOVA based on CytB and COI DNA regions revealed significant geographic differentiation between locations (i.e. across ~1500km) for all clades tested (Table 2.3), however these results must be interpreted with caution due to low sample size; particularly the structure detected within clades g4 and p2, for which $n \leq 6$. For clade p3 (the only clade with sufficient individuals collected at multiple sites within a region for statistical comparison) we found large and significant genetic differences between two sites on the East Coast separated by only 148km (Table 2.3), suggesting limited gene flow even on moderate spatial scales. Interestingly, the ITS2 dataset was generally less informative for understanding geographic relationships, producing much lower values of F_{ST} (Table 2.3).

Table 2.3: Geographical genetic structure within clades as determined by Analysis of Molecular Variance. F_{ST} values given with sample size for each location or site in parentheses, *p < 0.05, **p < 0.01, ***p < 0.001.

		F_{ST}		
	Clade	Cytochrome b	Cytochrome oxidase subunit I	Internal transcribed spacer 2
Locations compared				
East Coast, Tressler Bank	g1	0.480*** (n = 17, 9)	0.150* (n = 16, 8)	0.042 (n = 17, 8)
East Coast, Weddell Sea	g4	0.943** (n = 6, 5)	0.979** (n = 5, 5)	0.524* (n = 5, 3)
Antarctic Peninsula, Weddell Sea	p2	0.842* (n = 4, 4)	0.691* (n = 4, 5)	0.000 (n = 4, 5)
East Coast, Tressler Bank, Weddell Sea	p3	0.587*** (n = 24, 17, 3)	0.588*** (n = 14, 17, 6)	0.354*** (n = 16, 10, 5)
Sites within locations compared				
East Coast a, East Coast b	p3	0.775*** (n = 12, 4)	0.477*** (n = 8, 6)	0.006 (n = 18, 6)

2.5. DISCUSSION

Biodiversity in Antarctic benthic invertebrates is clearly underestimated. Analysis of mitochondrial and nuclear DNA sequences of the ecologically important and apparently circum-Antarctic amphipods *E. perdentatus* and *E. giganteus* has revealed the presence of several cryptic species within the genus. Separate consideration of these putative cryptic species shows that some may be endemic to specific regions but at least one may have a true circum-Antarctic distribution. However, preliminary observations of high genetic differentiation within widespread cryptic species, even on a scale < 200km, implies that populations are far from homogenous.

2.5.1. Molecular evidence of cryptic species

Our results challenge the morphological taxonomy of the common, giant Antarctic amphipods *Eusirus perdentatus* and *Eusirus giganteus* by providing strong molecular evidence of cryptic speciation, concordant across mitochondrial and nuclear markers (Figures 2.2 and 2.3). Species delimitation continues to be one of the most controversial issues in biological science (Sites & Marshall 2003). Nonetheless, several criteria are now considered to provide a valid argument for species boundaries based on genetic clades. The genealogical species concept (Baum & Shaw 1995) dictates that reciprocally monophyletic clades indicate true species, and the biological species concept (Mayr 1963) suggests that the existence of clades in sympatry indicates true reproductive isolation rather than simply geographic "races". Additionally, sequence divergence between clades should reflect values known to occur between morphologically-robust species and there should be a clear bimodal distribution of intraclade and interclade sequence divergence values (Held 2003), whilst the formation of unconnected haplotype networks in maximum parsimony analyses will also support species boundaries (Hart & Sunday 2007). Finally, these patterns should be concordant across several DNA regions to confirm that they do not simply reflect stochastic lineage sorting at one particular gene (see Avise 2000). The seven major clades detected in the present study (p1 – p3 within *E. perdentatus* and g1 – g4 within *E. giganteus*) meet every one of these criteria, indicating they represent seven cryptic species. Additionally, they satisfy a proposed amphipod COI species screening threshold (Witt *et al*., 2006), as well as the "4x" rule (Birky *et al.* 2005). However, it is likely that the true number of

cryptic species within this complex may be even greater, as rarefaction curves illustrated an overall undersampling of genetic diversity (Figure S2.1).

Although the divergence and monophyly of the seven clades were concordant across all three DNA regions, the mitochondrial regions CytB and COI created two unconnected haplotype networks within clade g4, which the nuclear region ITS2 formed as a single network (Figure 2.2). It is well-established that nuclear DNA is less sensitive to recent divergence than mitochondrial DNA, due to its four-fold larger effective population size (Wilson *et al.* 1985; Moore 1995; Barrowclough & Zink 2009). Therefore, we propose that the split within clade g4 identified by CytB and COI but not by ITS2 (Figure 2.2, clades g4a & g4b) may represent recent or ongoing speciation. Other potential explanations for the discrepancy include retention of ancestral polymorphisms or selection acting on mitochondrial loci – however these are unlikely as there was remarkable concordance between the two mitochondrial regions. This highlights the importance of comparing both nuclear and mitochondrial DNA regions to infer genetic patterns. Due to time and cost limitations there is current impetus to establish a single DNA region to act as a "barcode" for species delineation (Hebert *et al.* 2003a; Tautz *et al.* 2003). COI is by far the most popular DNA barcode candidate, particularly for crustaceans (Hebert *et al.* 2003b; Lefébure *et al.* 2006; Costa *et al.* 2007), although recent work advocates the ITS2 region (Yao *et al.* 2010). Interestingly, our results show that ITS2 was generally more effective in delimiting cryptic species in *E. perdentatus* and *E. giganteus* than COI, as it provided a larger gap between intra-clade and inter-clade sequence divergences (Figure 2.4). Nevertheless, some intragenomic variation limited the sample size we could use for our ITS2 dataset. This exemplifies the difficulties inherent in establishing a single molecular marker to effectively discriminate species.

This study provides a particularly strong demonstration of cryptic speciation in the Antarctic. Whilst numerous studies have reported evidence of cryptic speciation for Antarctic benthic taxa, many fail to compare between nuclear and mitochondrial patterns, and rarely do they meet all the criteria for species delineation mentioned above. Exceptions include the discovery of cryptic speciation in Antarctic ostracods using both COI and ITS (Brandão *et al*., 2010), in isopods using 16S and 18S (Raupach *et al*., 2007), and investigation of cryptic bivalve species using COI and 28S (Linse *et al*., 2007); although the two DNA regions were not congruent in the latter study. There are several Antarctic studies wherein species boundaries indicated by molecular data

have been subsequently confirmed by the discovery of fine, diagnostic morphological characters (e.g. Lörz *et al.* 2009; Janosik & Halanych 2010). Further work is now required to determine if the provisional cryptic species within *E. perdentatus* and *E. giganteus* are truly cryptic – i.e. possessing no discernable morphological differences – or if there are diagnostic features that have previously gone undetected. True cryptic speciation is common in marine organisms, as speciation in the sea is often more closely linked to non-visible traits such as chemical recognition systems than to morphological characters (Knowlton 1993). The existence of the proposed *Eusirus* cryptic species in sympatry implies that there must be some form of ecological niche separation between them (competitive exclusion principle: Hardin 1960). Prey specialization has been identified as a likely form of niche separation for Antarctic amphipods (Watling & Thurston 1989; Dauby *et al.* 2001).

2.5.2. Genetic diversity and structure within cryptic *Eusirus* species

Our study revealed highly significant genetic differentiation among several locations (> 1500km apart), and between two sites (148km apart) for four cryptic *Eusirus* species, suggesting limited dispersal among geographically isolated populations. We acknowledge that low and unbalanced sample sizes for AMOVA may have limited the power of our analyses, and the results should therefore be regarded with caution. This highlights the difficult nature of Antarctic research, where the re-sampling of field sites (e.g. after the discovery of cryptic species) is often logistically infeasible; as it was for the present study. However, the most likely effect of low sample size is a reduction in the ability to detect population differentiation (Waples 1998; Björklund & Bergek 2009); yet majority of our results indicated high differentiation ($F_{ST} > 0.4$), significant at $p < 0.01$, and are therefore unlikely to reflect a "false positive".

Population structure was much more apparent for the mitochondrial DNA regions than for ITS2, which may reflect the increased sensitivity of mitochondrial DNA to recent genetic subdivision, as discussed above. Alternatively, it may reflect the maternal inheritance of mitochondrial DNA. If dispersal is male-biased, mitochondrial regions may display signatures of differentiation where biparentally inherited nuclear DNA regions do not (Petit & Excoffier 2009). However, ITS2 did reveal significant

geographic differentiation for two of the clades, suggesting that sex-biased dispersal is unlikely for *Eusirus* species.

Restricted gene flow over the geographic scales we investigated is not surprising for benthic, brooding organisms due to their limited capacity for dispersal. Regardless, this study provides one of the first examples of highly restricted intraspecific gene flow within the eastern Antarctic benthic environment (F_{ST} based on COI = 0.48, $p < 0.001$). Whilst Arango *et al.* (2010) explored genetic structure in pycnogonids on an even finer scale in the eastern Antarctic, genetic differentiation between populations was considerably weaker (F_{ST} based on COI = 0.07, $p < 0.05$). Most other studies that address genetic population structure within Antarctic regions are restricted to the Antarctic Peninsula and Scotia Arc, where gene flow is influenced by a fragmented topography and numerous converging oceanographic currents (e.g. Rogers *et al.* 1998; Page & Linse 2002; Hoffman *et al.* 2010). Only a handful of studies have explored benthic intraspecific gene flow around the Antarctic continent (e.g. bivalve: Linse *et al.* 2007; amphipod: Lörz *et al.* 2009; pycnogonid: Arango *et al.* 2010), as most genetic subdivision at this broader scale reflects cryptic speciation. Within *Eusirus* clades, we found gene flow on a circum-Antarctic scale to be so highly restricted (Table 2.3) that future allopatric speciation within these clades is likely. As mentioned previously, clade g4 already appears to be undergoing allopatric speciation, with all specimens from the Weddell Sea forming a distinct group (Figure 2.2, clade g4b).

Reduced gene flow in brooding organisms has long been thought to facilitate their speciation (Gooch 1975; Crisp 1978; Cohen & Johnston 1987). In the Antarctic, brooding is invoked to explain why groups such as peracarid crustaceans are particularly speciose (Brandt 1999; De Broyer *et al.* 2003b; Held & Leese 2007). Over the spatial scales we explored for cryptic *Eusirus* species, geographic distance alone could be sufficient to generate the genetic differentiation observed, although the spatial heterogeneity of Antarctic benthic habitat is also thought to play a role in allopatric speciation (De Broyer *et al.* 2003b; Rogers 2007). Bathymetric separation is also likely to restrict gene flow within peracarid crustacean species (France & Kocher 1996; Held & Wägele 2005). Given the eurybathic distribution of the *Eusirus* species, it was a goal of the present study to assess intraspecific gene flow with depth, however the discovery of so many cryptic species reduced intraspecific sample size to a level that prohibited such tests. This highlights the need for intensified sampling over an increased depth range.

In contrast to the geographic genetic structure determined by AMOVA, there is also evidence of remarkable genetic homogeneity over large distances in the *Eusirus* species complex. For example, haplotypes within clade g2 are shared between the Antarctic Peninsula and Ross Sea (~5000km). It is highly unlikely that this represents homoplasy, given that the same individuals from each location share identical haplotypes for all three of the DNA regions investigated (Figure 2.2). Large scale genetic homogeneity in benthic brooders has become an increasingly common discovery in the Antarctic (e.g. Hunter & Halanych 2008; Wilson *et al.* 2009; Krabbe *et al.* 2010) – this study now adds perhaps one of the most extreme examples. Authors have postulated a role for passive long-distance rafting via floating substrata or anchor ice as a mechanism for maintaining this remarkable homogeneity. This is a possibility for *Eusirus*, given that *E. perdentatus* (sensu lato) has been observed associated with epibenthic substrate (De Broyer *et al.* 2001). However, we cannot dismiss that genetic homogeneity may simply represent the retention of ancestral polymorphisms. Regardless, this presents a very different circumstance to the marked geographic differentiation observed in other clades. Although seemingly counterintuitive, disparate genetic structure in closely related species has been observed in other taxa (Marko 2004; Wilson *et al.* 2007), and may reflect processes specific to high latitudes.

2.5.3. Speciation and dispersal in Antarctic benthos

The high species diversity of the Antarctic benthic environment is now widely believed to reflect historical cycles of glaciation acting as a "diversity pump" (Clarke & Crame 1992; Clarke 1996). When glacial sheets were grounded on the continental shelf, benthic taxa are proposed to have survived in physically isolated ice-free refugia, which allowed an allopatric fixation of genetic differences over time (summarised in Thatje *et al.* 2005). If conditions such as habitat and prey choice differed between refugia, genetic differentiation could have been so great to cause reproductive isolation. Following glacial retreat, the re-establishment of these differentiated populations on the shelf provides a worthy explanation for the numerous sympatric, morphologically cryptic "species complexes" observed for a number of endemic Antarctic groups (e.g. Held & Wägele 2005; Raupach & Wägele 2006; Hunter & Halanych 2008; Mahon *et al.* 2008; Wilson *et al.* 2009), and now also for *Eusirus*.

Cryptic species revealed through genetics are often found to have only a partial geographic distribution of the morphologically-determined species they constitute. This has fostered the argument that true circum-Antarctic distributions are in reality unlikely for benthic fauna, and may simply represent aggregations of undetected, geographically restricted cryptic species (Raupach *et al.* 2007; Lörz *et al.* 2009; Brandão *et al.* 2010). However, clade p3 from this study provides evidence to the contrary, demonstrating the occurrence of a single cryptic amphipod species at five locations spread broadly around the Antarctic coast (Figure 2.2). This presents a strong case for circum-Antarctic distribution; other taxa have been claimed to be circum-Antarctic from similar or even less extensive records of occurrence (Wägele 1987a; Barnes & Peck 1997; Raupach *et al.* 2010). Recently, Allcock *et al.* (2010) screened for cryptic species in benthic *Pareledone* octopus, and provided strong evidence that *P. aequipapillae* was a single, truly circum-Antarctic species; as did Arango *et al.* (2010) for the brooding pycnogonid *N. australe*. Assumptions that benthic species cannot maintain true circum-Antarctic distributions should therefore be revised.

Decreased intraspecific genetic variation and structure have been postulated as high latitude phenomena resulting from glacial cycles, based largely on observations from the northern hemisphere (Hewitt 1996; Martin & McKay 2004; Maggs *et al.* 2008). In contrast, our study reveals high levels of genetic diversity and strong geographic genetic structure within several cryptic *Eusirus* species (Tables 2.2 and 2.3). Brandt *et al.* (2007b) and Wilson *et al.* (2009) have also argued that the Antarctic benthos may not conform to a latitudinal cline in diversity as generalised from the northern hemisphere. Rather, an emerging pattern from this and previous studies (Marko 2004; Wilson *et al.* 2007) is that high latitude genetic diversity and structure can be unpredictable and contrasting – even between sibling species with the same presumed dispersal capacities (e.g. genetic homogeneity in *Eusirus* clade g2 versus strong differentiation in clade p3). A potential explanation is that various different refugia were available for benthic survival during Antarctic glaciation, resulting in different post-glacial recolonisation processes, and ultimately different genetic signatures (Thatje *et al.* 2005).

2.5.4. Concluding remarks

Our results present strong evidence that *E. perdentatus* harbours two previously undetected cryptic species, and *E. giganteus* harbours at least three, revealing considerable levels of otherwise unknown biodiversity in Antarctic benthic invertebrates. Importantly, the *Eusirus* species complex studied herein comprises some of the largest amphipods known on the Antarctic shelf (Klages 1993; De Broyer *et al.* 2003a), so if we have failed to recognise the diversity of these giants until now, where does this leave the vast majority of much smaller amphipods and invertebrates in general? Furthermore, rarefaction techniques employed in this and a previous study (Wilson *et al.* 2009) have indicated that genetic diversity in the Antarctic will continue to be underestimated until the extent and intensity of sampling are increased. The Antarctic marine environment is predicted to experience rapid and significant effects from climate change hence there is an urgent need to document its biodiversity before species are lost. Fortunately, measures are in place to increase molecular barcoding research, but with less than 1% of Antarctic marine invertebrates currently barcoded (Grant & Linse 2009) there is still a long way to go, and as we have illustrated, data from multiple gene regions will more accurately reflect speciation processes. Our study also emphasises the importance of exploring intraspecific genetic diversity and structure – which for the Antarctic benthos, appears unpredictable. Improving our knowledge of benthic population structure and connectivity will benefit the design of Marine Protected Areas, which have recently been endorsed to conserve Antarctic biodiversity (CCAMLR 2005). For several *Eusirus* species at least, high geographical isolation of populations suggests that management efforts will need to be widespread in order to maintain the genetic integrity, and therefore evolutionary potential, of the species. Continued molecular research is imperative to enhance our understanding of the diversity, distribution and dispersal of Antarctic benthic fauna, and ultimately, how best to conserve this environment.

Chapter 3. Genetic population structure in the Antarctic benthos: insights from the widespread amphipod, *Orchomenella franklini*

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3.1. ABSTRACT

Currently there is very limited understanding of genetic population structure in the Antarctic benthos. We conducted one of the first studies of microsatellite variation in an Antarctic benthic invertebrate, using the ubiquitous amphipod *Orchomenella franklini.* Seven microsatellite loci were used to assess genetic structure on three spatial scales: sites (100s of metres), locations (1-10 kilometres) and regions (1000s of kilometres), sampled in East Antarctica at Casey and Davis stations. Considerable genetic diversity was revealed, which varied between the two regions, and also between polluted and unpolluted sites. Genetic differentiation among all populations was highly significant (F_{ST} = 0.086, R_{ST} = 0.139, p < 0.001), consistent with the brooding mode of development in *O. franklini*. Hierarchical AMOVA revealed that the majority of the genetic subdivision occurred across the largest geographical scale, with $N_em \approx 1$ suggesting insufficient gene flow to prevent independent evolution of the two regions, i.e. Casey and Davis are effectively isolated. Isolation by distance was detected at smaller scales and indicates that gene flow in *O. franklini* occurs primarily through stepping-stone dispersal. Three of the microsatellite loci showed signs of selection, providing evidence that localised adaptation may occur within the Antarctic benthos. These results provide insights into processes of speciation in Antarctic brooders, and will help inform the design of spatial management initiatives recently endorsed for the Antarctic benthos.

3.2. INTRODUCTION

Gene flow – or genetically effective migration – is one of the most important factors governing the evolution of species (Slatkin 1994; Bohonak 1999; Morjan & Rieseberg 2004). Gene flow can dampen localised adaptation yet spread advantageous alleles for the cohesive evolution of the species (e.g. in the face of climate change), whereas the absence of gene flow can lead to population divergence, and ultimately speciation (Levins 1964; Gooch 1975; Slatkin 1987). Anthropogenic impacts have the potential to disrupt gene flow and alter genetic diversity of natural populations (e.g. Allendorf *et al.* 2008), thereby affecting the evolutionary potential of species. Understanding the genetic structure of populations will therefore shed light on how species may respond to these impacts. Moreover, genetic studies can help optimise the design of conservation efforts to preserve genetic diversity, in order to help ensure the long-term adaptability and persistence of species (Bowen 1999; Bell & Okamura 2005).

Marine fauna are currently threatened by a plethora of human activities including fisheries harvest, habitat destruction, localised pollution, introduced species and climate change (see Gray 1997; Costello *et al.* 2010). Initiatives such as Marine Protected Areas (MPAs) are one of the most important tools for the conservation of marine populations, the success of which relies on 'spillover' of individuals from protected areas to replenish outside populations (Agardy 1994; Lubchenco *et al.* 2003; Sale & Kritzer 2003). Estimates of genetic connectivity thus help determine the optimal size and placement of MPAs to achieve this desired broad-scale flux (Palumbi 2003; Shanks *et al.* 2003). There is strong evidence of a correlation between the dispersal capacity inferred by a species' pelagic larval phase and the genetic connectivity of populations (and subsequent potential for MPA connectivity), however exceptions are common, and patterns of marine genetic structure are far from predictable (reviewed in Palumbi 1995; Féral 2002; Miller & Ayre 2008). Furthermore, local adaptation has been increasingly emphasised for its role in structuring marine populations (e.g. Hedgecock 1986; Hilbish 1996; Sotka 2005). Local adaptation may be particularly important in the face of localised marine pollution, which can alter allele frequencies or genetic diversity in exposed populations (e.g. Battaglia *et al.* 1980; Ma *et al.* 2000; De Wolf *et al.* 2004), in turn affecting speciation processes and ultimately, species fitness (Bickham *et al.* 2000).

The Antarctic benthos represents one of the most isolated marine ecosystems on the planet, with particularly unique fauna (reviewed in Picken 1985; Arntz *et al.* 1994) that are considered vulnerable to future environmental change (Peck 2005). Intraspecific genetic structure in Antarctic benthic organisms is poorly understood, and the need for genetic research has been highlighted (Thatje *et al.* 2005; Held & Leese 2007; Wilson *et al.* 2007; Hoffman *et al.* 2010). This is particularly pertinent given a recent endorsement by the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) to establish a network of MPAs in Antarctica (CCAMLR 2005). Determining the location and size of these MPAs is still in progress and is hindered by a lack of existing baseline knowledge on Antarctic marine fauna (Harris *et al.* 2007). Improving our understanding of benthic gene flow and genetic diversity in Antarctica will not only help inform the design of these MPAs, but will also shed light on the high rates of speciation prevalent in Antarctic benthos (Thatje *et al.* 2005; Held & Leese 2007; Leese & Held 2008). Furthermore, studying these microevolutionary processes will help predict the potential for Antarctic organisms to adapt to existing threats, such as local pollution surrounding human settlements (Lenihan *et al.* 1990; Clarke & Harris 2003), and broad-scale climate change (Meredith & King 2005).

Studies of gene flow in Antarctic benthic fauna have focused primarily on large-scale connectivity over major hydrographic features such as the Polar Front, or abyssal depths between islands (e.g. Linse *et al.* 2007; Hunter & Halanych 2008; Wilson *et al.* 2009). Commonly, these studies have revealed highly distinct genetic lineages assumed to represent cryptic species (see Janosik & Halanych 2010 for recent summary). This partly explains why truly *intra*specific genetic patterns remain much less explored. What has emerged from the limited population-level studies is that the unique hydrography of Antarctica may have an important influence on genetic structure. For instance, local circulation patterns are believed to play a role in isolating populations of species that display surprisingly fine-scale (< 20km) genetic subdivision, despite possessing pelagic larvae for dispersal (icefish: Clement *et al.* 1998; scallop: Guidetti *et al.* 2006). Other unique mechanisms such as iceberg scouring and historical glaciation have been implicated when populations from different locations exhibit markedly different levels of genetic diversity (e.g. amphipod: Baird *et al.* 2011; ascidian: Demarchi *et al.* 2010; isopod: Leese *et al.* 2010, pycnogonid: Arango *et al.* 2010).

Brooding benthic organisms are particularly interesting candidates in which to address questions of gene flow and speciation in Antarctica, as their lack of a pelagic

dispersal phase should lead to high genetic structuring of populations (De Broyer *et al.* 2003b; Rogers 2007). In Antarctica, brooding taxa are highly speciose and largely endemic, with several groups that have undergone intense radiation (Watling & Thurston 1989; Clarke & Crame 1992; Brandt 1999, 2005). One such group is the amphipods, which are remarkably abundant crustaceans that occupy a wide range of ecological niches and play a significant role in Antarctic trophic exchanges (Jażdżewski *et al.* 1991; Dauby *et al.* 2001; De Broyer *et al.* 2003a; De Broyer *et al.* 2003b). We chose to study the ubiquitous amphipod *Orchomenella franklini* to address the current paucity of knowledge on intraspecific genetic structure in Antarctic benthos. *O. franklini* often dominates Antarctic shallow water communities (Tucker & Burton 1988; Stark 2000; Stark *et al.* 2004; Knox 2007), and its presence in polluted bays adjacent to Antarctic research stations allowed us to investigate potential effects of contamination on genetic diversity.

We used microsatellite markers to investigate genetic variation in *O. franklini*, as the high variability of microsatellites enables resolution of genetic structure over fine (< 100km) spatial scales (Wright & Bentzen 1994; Jarne & Lagoda 1996; Parker *et al.* 1998; Goldstein & Schlötterer 1999; Sunnucks 2000; Held & Leese 2007), which in Antarctica remain the least understood (Held & Leese 2007; Krabbe *et al.* 2010). To our knowledge just a single study to date has used microsatellites to explore gene flow in an Antarctic benthic invertebrate, and this focused on large-scale migration between islands (Leese *et al.* 2010). Thus we present the first known Antarctic study of microsatellite variation in a benthic invertebrate over small (100s of metres), moderate (10s of kilometres), and large (1000s of kilometres) spatial scales.

3.3. METHODS

3.3.1. Sampling

Samples of *Orchomenella franklini* were collected from two geographical regions, adjacent to the Australian research stations Casey (66ºS, 110ºE) and Davis (68ºS, 78º´E), in East Antarctica. Casey and Davis are separated by approximately 1400km (Figure 3.1), and were sampled during the summer months of 2009 and 2010 respectively. Within the Casey region samples were collected at eight locations (~1–

30km apart; Figure 3.1). Within the Davis region samples were collected at four locations (~3–20km apart; Figure 3.1). Locations were classified as either polluted or unpolluted (Figure 3.1, Table S3.1[1]) based on proximity to known contaminated areas (Deprez *et al.* 1999; Stark *et al.* 2003a; Stark *et al.* 2011), and knowledge of the extent of dispersion of these contaminants (Howington *et al.* 1992; Stark *et al.* 2011), which include hydrocarbons, heavy metals and faecal sterols. Within each location two to four sites were sampled 100m apart, except in two instances where only a single site was accessible due to local ice conditions (Table S3.1). At each site a van-veen grab was used to take a small (< $1m^3$) sample of the benthic sediment from no more than 10m water depth. This sediment was sieved on a 0.5mm mesh and retained fauna were sorted under a dissecting microscope. All identified *O. franklini* specimens were removed and stored at 4°C in vials of 80% ethanol (see Table S3.1 for final sample sizes). All necessary permits were obtained for the described field studies from the Commonwealth of Australia under the Antarctic Marine Living Resources Act 1981. Collections from Casey were made under permit AMLR 08-09-3051 and collections from Davis were made under permit AMLR 09-10-3051.

3.3.2. Development of microsatellite loci

A genomic library of *O. franklini* was made by ecogenics Gmbh (Zurich, Switzerland) based on a pooled sample of DNA from 15 individual amphipods. SNX forward and reverse linkers were ligated onto size-selected DNA following the procedure of Hamilton *et al.* (1999) and these were enriched for $(TAC)_{10}$, $(AAC)_{10}$, $(GT)_{13}$, $(CT)_{13}$ and $(ACAG)_7$ oligonucleotide repeats by magnetic bead selection (Gautschi *et al.* 2000a; Gautschi *et al.* 2000b). The enriched library was cloned and 1406 recombinant colonies screened for the presence of microsatellites by hybridisation. DNA inserts from 230 positive clones were subsequently sequenced and primers were designed for 23 microsatellite loci and tested for polymorphism. Seven loci (Table 3.1) were considered suitable for population-level analysis.

[1] All supplementary figures and tables for this chapter are provided in Appendix II.

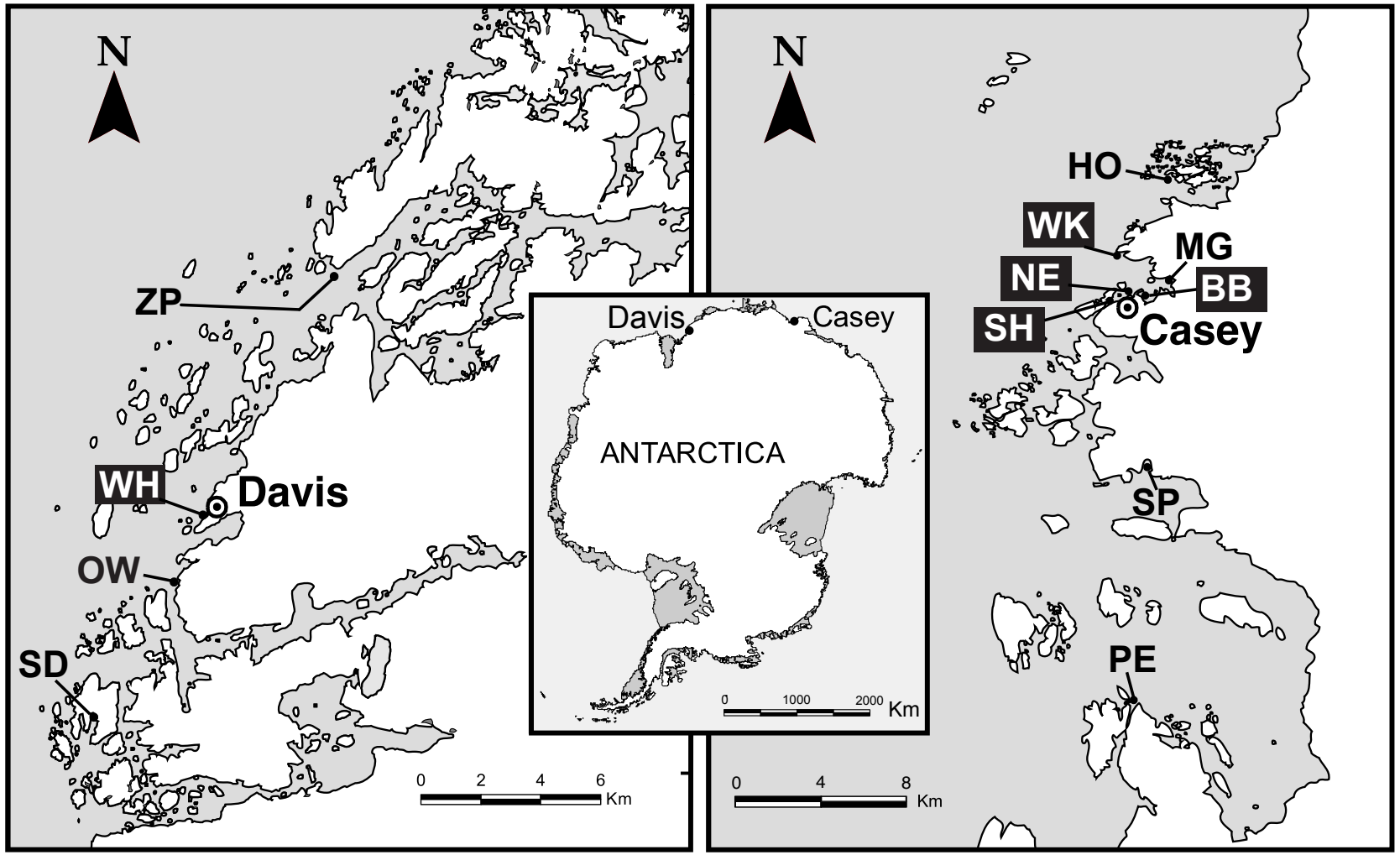

Figure 3.1: Map showing the positions of the Casey and Davis regions in East Antarctica, with a close-up of each region showing the locations sampled for *O. franklini*. Within each location 1 to 4 replicate sites were sampled (see Table S3.1 for region, location and site details). Dark boxes indicate polluted locations. Images derived from base maps courtesy of the Australian Antarctic Data Centre.

Table 3.1: Details for the seven microsatellite loci amplified in *O. franklini*. PCR thermal protocols and corresponding primer details are given for each multiplex reaction. Fluorescent dyes were used to label the forward primers. The number of alleles provided is the total observed across all individuals.

Multiplex	PCR thermal protocol	Locus	Primer sequence (5'-3')	Flourescent dye	Repeat motif	Total no. of alleles	Allele size range (bp)
A	15 min at 95˚C [30s at 94˚C, 90s at 58.5˚C, 60s at 72˚C] x31 30 min at 72˚C	*Orcfra4*	F: AGCAGTCCCTAACAGAAATGG R: GGCGCTCCAATAAGTTCTTC	D2	$(AAC)_7$	9	97 - 116
		Orcfra5	F: GTGGGGGCTACGGTAGAAAC R: TTGTTTGTATTGCTCTTGTAACTATTG	D3	$(CAA)_7$	6	138 - 159
B	15 min at 95˚C [30s at 94˚C, 90s at 61.5˚C, 60s at 72˚C] x31 30 min at 72˚C	*Orcfra3*	F: AAACACAGCCCCAGTTGATG R: TACCATCCCAGGACCACAAG	D2	$(CAA)_9$	8	228 - 249
		Orcfra13	F: AGATGCTGTATTATACTCGTGCTG R: CGATCTGCAACATAAACAACAAC	D3	$(TGT)_6$	7	113 - 128
		Orcfra26	F: CGAGCCTGTGCACTCCTAC R: CGGTGGATAGTTGTTCATGC	D4	$(CA)_4GA(CA)_7$	8	157 - 173
C	15 min at 95˚C [30s at 94˚C, 30s at 55˚C, 60s at 72˚C] x40 10 min at 72˚C	*Orcfra6*	F: TGTAGACATCACTGCTGGTTAGG R: TCGTTTTGCATCAAGACCAC	NED	$(CTA)_6$	18	86 - 102
		Orcfra12	F: CCGGGGTTCTATGAATTACC R: AGCGCTAAGTGGTGATGAAG	FAM	$(CTAC)_{21}$	24	197 - 237

3.3.3. Microsatellite genotyping

Whole specimens of *O. franklini* were used for DNA extractions due to small body size (2-8 mm). DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN) according to the manufacturer's instructions, with elution volume decreased to 120µl to maximise DNA concentration. Microsatellite loci were amplified in three multiplex polymerase chain reactions (PCRs) (Table 3.1). For multiplex reactions A and B, 20µl reactions using the QIAGEN Multiplex PCR kit were used, with a final concentration of 1x Qiagen Multiplex PCR Master Mix (provides 3mM MgCl and 1 unit HotStar Taq DNA Polymerase), 0.2µM each primer (forward primers were fluorescently labeled), and approximately 100ng template DNA. Capillary separation of amplified fragments occurred on an automated sequencer (CEQ 8000, Beckman Coulter), and alleles were scored (according to PCR fragment size) with CEQ 8000 Genetic Analysis System software version 8.0. Multiplex C was amplified at the Australian Genome Research Facility, with capillary separation occurring on an Applied Biosystems 3730 DNA Analyser and alleles scored using Applied Biosystems GeneMapper software version 3.1. All fragment data were visually checked for allele scoring errors and stutter. Micro-checker 2.2.3 (Van Oosterhout *et al.* 2004) was used to check data for the presence of null alleles. Loci were tested for linkage disequilibrium in Genepop 4.0.10 (Raymond & Rousset 1995) with the critical level $p < 0.05$ adjusted for multiple comparisons, using the sequential Bonferroni procedure (Rice 1989).

3.3.4. Genetic diversity of populations

Measures of genetic diversity, including observed heterozygosity (H_O), unbiased expected heterozygosity (H_E) and allelic richness (standardised for sample size: A_R) at each locus were calculated in FSTAT 2.9.3 (Goudet 1995). To determine whether diversity measures differed between Casey and Davis, and among polluted and unpolluted sites, permutation tests were performed on H_O, H_S and A_R in FSTAT. The number of private alleles (P_A) at each site was determined using Genalex 6.41 (Peakall & Smouse 2006). There is evidence that the occurrence of private alleles most closely follows a Poisson distribution (Chakraborty & Griffiths 1982), so we used a Poisson generalised linear model to assess the effect of region and pollution on P_A, carried out in R 2.12.2 (R Development Core Team 2011).

Exact tests (using default Markov chain parameters, performed in Genepop) were used to test for departures from Hardy-Weinberg Equilibrium, with Wright's fixation index (F_{IS}) for each locus-site combination used to determine the nature of those departures (where $F_{IS} < 0$ indicates heterozygote excess and $F_{IS} > 0$ indicates heterozygote deficits). Significance levels were adjusted for multiple comparisons using the sequential Bonferroni procedure (Rice 1989).

Cryptic species are common in Antarctic benthic invertebrate fauna (Rogers 2007), in part because many taxa are poorly studied. To check for the presence of cryptic species within *O. franklini*, Principal Coordinate Analysis (PCoA in Genalex) was used to examine the complete multilocus data set for evidence of distinct genetic groups (Boissin *et al.* 2008; Reeves & Richards 2011).

3.3.5. Population differentiation

We calculated Weir and Cockerham's F_{ST} estimates (Weir & Cockerham 1984) to examine genetic differentiation among all sites. Since F_{ST} assumes an infinite allele model of mutation (IAM), we also calculated R_{ST} which assumes a stepwise mutation model (SMM). Currently there is no consensus over which model is more appropriate for microsatellite data, so the conservative approach is to calculate both (Balloux & Lugon-Moulin 2002). F_{ST} and R_{ST} were calculated in Arlequin 3.11 (Excoffier *et al.* 2005), using 50000 permutations to assess significance. The high variability of microsatellites can result in depressed estimates of F_{ST}, therefore we also calculated '$F`_{ST}$' (a standardised measurement which accounts for high within-population variation), using RECODEDATA 0.1 (Meirmans 2006). Matrices of the pairwise differentiation (as both F_{ST} and R_{ST}) between all sites were generated in Genepop.

The partitioning of genetic variation among regions, locations nested within regions, sites nested within locations and individuals nested within sites was determined using a 4-level hierarchical analysis of molecular variance (AMOVA). AMOVA was performed using Hierfstat (Goudet 2005), which calculates variance components and *F*-statistics (Weir & Cockerham 1984) for each hierarchical level according to Yang (1998). Departures from values expected under panmixis (i.e. $F_{ST} = 0$) at each hierarchical level were determined with 10,000 permutations of the data. We estimated the migration occurring between Casey and Davis as $N_e m = 1/4(1/F_{ST} - 1)$ (Wright 1951), using the F_{ST} among regions generated from hierarchical AMOVA. F_{ST} varied

considerably among loci (see Section 3.4.2.), so we tested for evidence of selection at each of the seven loci using Lositan (Antao *et al.* 2008). Confidence intervals (99%) for neutral loci were determined using 20,000 simulations and the recommended 'neutral mean F_{ST}' option (Hemond & Wilbur 2011).

Coastal marine populations are unlikely to disperse according to the island model (Hellberg *et al.* 2002) so we tested for evidence of isolation by distance (which indicates a stepping-stone mode of dispersal: Kimura 1953) among *O. franklini* populations within the Casey and Davis regions. We examined the relationship between geographic distance (as the natural logarithm of the estimated shortest water-based route) and genetic differentiation (as linearised F_{ST}; i.e. $F_{ST}/(1-F_{ST})$) between all sites within each region, using Mantel tests implemented in Genepop. Mantel tests were also performed assuming the SMM (i.e. using $R_{ST}/(1-R_{ST})$ for genetic differentiation estimates). Data were permuted 10,000 times to determine significance.

3.4. RESULTS

3.4.1. Genetic diversity of populations

A total of 718 *Orchomenella franklini* specimens were genotyped for the seven microsatellite loci (448 from Casey, 270 from Davis: Table S3.1). None of the loci showed evidence of linkage disequilibrium ($p > 0.05$ for all pairwise comparisons). The number of alleles observed at each locus ranged from 6 to 24 (Table 3.1). Average allelic richness, observed heterozygosity and expected heterozygosity were all significantly higher at Davis ($A_R = 4.46$, $H_O = 0.495$, $H_E = 0.574$), compared to Casey ($A_R = 3.62$, $H_O = 0.428$, $H_E = 0.447$; $p < 0.001$; Table 3.2). None of these diversity measures were found to differ significantly among polluted and unpolluted sites within either of the regions.

Table 3.2: Genetic diversity of *O. franklini* populations from Casey and Davis. Allelic richness (A_R), expected heterozygosity (H_E), and observed heterozygosity (H_o) are given for each microsatellite locus, and averaged over all loci.

	Casey			Davis		
	A_R	H_E	H_O	A_R	H_E	H_O
Orcfra3	4.655	0.730	0.709	4.768	0.691	0.663
Orcfra4	1.848	0.118	0.089	3.705	0.630	0.230
Orcfra5	1.547	0.081	0.053	2.404	0.197	0.128
Orcfra6	6.009	0.814	0.795	5.409	0.645	0.584
Orcfra12	6.975	0.812	0.766	9.287	0.895	0.900
Orcfra13	2.963	0.533	0.542	3.066	0.522	0.541
Orcfra26	1.338	0.040	0.041	2.567	0.438	0.422
Overall	3.619	0.447	0.428	4.458	0.574	0.495

A total of 16 private alleles were observed across the entire dataset (Table S3.2). Standard diagnostics revealed that the Poisson generalised linear model was an appropriate model for the number of private alleles per population (P_A), with no evidence of overdispersion. The model revealed strong evidence that P_A was significantly greater at Davis than at Casey ($p = 0.002$), and also significantly greater at unpolluted sites compared to polluted sites ($p = 0.005$; Table S3.2). There was no evidence of a significant interaction between the two factors ($p = 0.307$), and average sample size was almost identical for unpolluted and polluted populations, thus could be disregarded as a potential confounding factor.

Most populations were in Hardy-Weinberg Equilibrium: of the 175 tests across all loci by all sites, only eight were significant after Bonferroni correction (Table S3.2). For just one of these significant departures from HWE did the F_{IS} value represent an excess of heterozygotes (at locus *Orcfra13* for site NEa at Casey; Table S3.2). The remaining seven were heterozygote deficits at locus *Orcfra4* (Table S3.2), and all of these occurred at Davis sites. Heterozygote deficits can result from the presence of null alleles; indeed at *Orcfra4* we found evidence of null alleles for all nine sites at Davis, and for three of the 16 sites at Casey. We subsequently adjusted allele frequencies at *Orcfra4* to account for the presence of null alleles (using the Oosterhout correction algorithm in Microchecker), but this made no difference to the significance of genetic differentiation or isolation by distance determined under either the IAM or SSM, therefore results for the raw data alone are presented for simplicity. Evidence of null alleles was also detected at *Orcfra5* (in 5 sites), and *Orcfra6* (in 3 sites). Adjusting

allele frequencies at these loci was considered unnecessary because the null alleles were only detected in a small proportion of the total 25 sites.

There was no evidence of cryptic species within the samples of *O. franklini* from Casey and Davis. Although PCoA explained 67% of the variation in the multilocus genetic data within the first three co-ordinates, it indicated just a single genetic group within the sample (Figure S3.1).

3.4.2. Population differentiation

Genetic differentiation among all sites was highly significant, regardless of the mutational model assumed (F_{ST} = 0.086, p < 0.001; R_{ST} = 0.139, p < 0.001; F'_{ST} = 0.162; Table 3.3). Hierarchical AMOVA revealed that the majority of this genetic differentiation occurred between Casey and Davis (69%), with significant differentiation also occurring among locations within each region (2%), but not among sites within each location (Table 3.4). A considerable amount of the variation (29%) was also due to differences among individuals within each site (Table 3.4). This hierarchical structure was also reflected in pairwise differentiation estimates: F_{ST} between Casey sites and Davis sites ranged from 0.120 to 0.199, whereas pairwise F_{ST} estimates among all sites within Casey and Davis ranged from 0 to 0.031 (Table S3.3). Pairwise R_{ST} estimates were consistently higher than F_{ST} values, but showed the same pattern; ranging from 0.063 to 0.486 between Casey and Davis, from 0.000 to 0.119 within Casey, and from 0.000 to 0.129 within Davis (Table S3.3). The estimate of migration (N_em) occurring between Casey and Davis was 1.4 (both before and after the removal of loci under selection; see below).

Locus *Orcfra4* produced the highest overall F_{ST} values (Table 3.3), and we found evidence that this locus was under directional selection (as indicated by 99% confidence intervals). We also detected balancing selection at the two loci which produced the lowest overall F_{ST} estimates: *Orcfra5* and *Orcfra12* (Table 3.3). We subsequently removed these three loci from the analyses, but the results remained unchanged (Tables 3.3 & 3.4). Interestingly, F_{ST} estimates for the differentiation of locations within regions were not particularly high for *Orcfra4*, nor were they particularly low for *Orcra5* and *Orcfra12*. Indeed, when we tested loci for evidence of selection within the Casey and Davis datasets independently, all loci were found to be neutral. This suggests that selection associated with *Orcfra4*, *Orcfra5* and *Orcfra12* is

occurring at the regional scale (i.e. between Casey and Davis) but not on a smaller scale (i.e. between populations within each of the regions).

We found evidence of isolation by distance indicative of stepping-stone dispersal among sites within both the Casey and Davis regions. Mantel tests indicated a significant correlation between genetic and geographic distance under both the IAM (Casey: p = 0.002; Davis: p = 0.000; Figure 3.2a), and the SMM (Casey: p = 0.003; Davis: p = 0.019; Figure 3.2b).

Table 3.3: Estimates of genetic differentiation (F_{ST}, R_{ST} and $F`_{ST}$) among all sites for *O. franklini*, given for each locus and over all loci. The overall estimate of F_{ST} excluding loci potentially under selection (*Orcfra4*, *Orcfra5* and *Orcfra12*) is provided in parentheses. Negative values have been converted to zero. Significance of differentiation is indicated as ***p<0.001.

	F_{ST}	R_{ST}	$F`_{ST}$
Orcfra3	0.091	0.177	0.254
Orcfra4	0.206	0.234	0.205
Orcfra5	0.012	0	0.013
Orcfra6	0.056	0.027	0.146
Orcfra12	0.049	0.147	0.128
Orcfra13	0.089	0.133	0.159
Orcfra26	0.154	0.161	0.190
Overall:	0.086*** (0.085***)	0.139***	0.162

Table 3.4: The partitioning of genetic variation in *O. franklini* at each spatial level as indicated by hierarchical AMOVA**.** Results of the analysis excluding loci potentially under selection (*Orcfra4*, *Orcfra5* and *Orcfra12*) are also provided. Negative values have been converted to zero. Significance is indicated as *p<0.05, **p<0.01, ***p<0.001.

	Over all loci			Excluding potentially selected loci		
	F-statistic	var. component	% variance	F-statistic	var. component	% variance
Among regions	0.156***	0.640	68.7	0.152***	0.393	86.4
Among locations within regions	0.005**	0.019	2.0	0.006**	0.014	3.0
Among sites within locations	0	0	0	0	0	0
Within sites	0.079	0.273	29.3	0.002	0.048	10.6

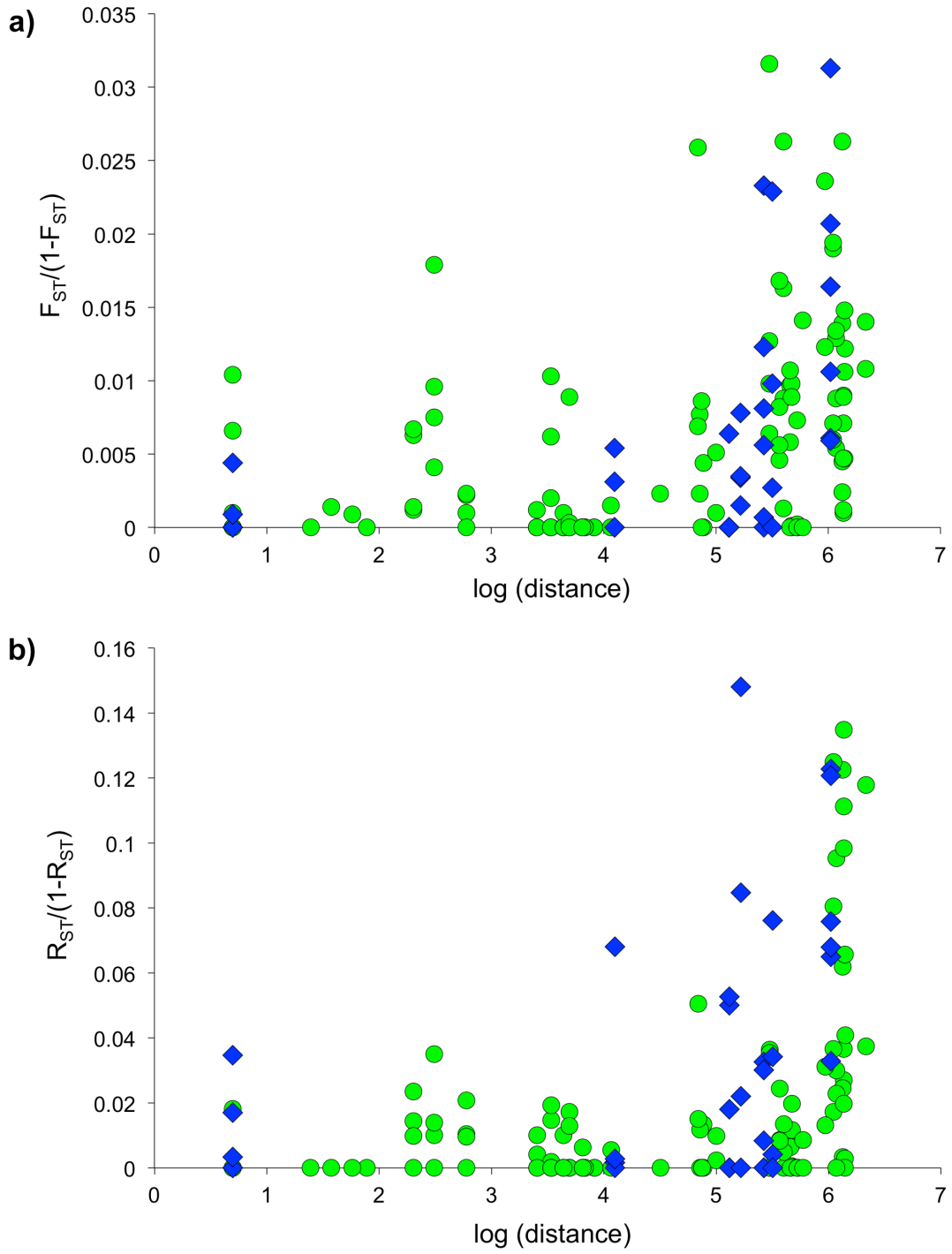

Figure 3.2: The relationship between geographic distance and a) linearised F_{ST}, and b) linearised R_{ST}, for *O. franklini*. In both cases, genetic isolation by distance was significant (see Section 3.4.2.). Green circles represent data from Casey; blue diamonds represent data from Davis.

3.5. DISCUSSION

This study has revealed considerable genetic diversity and population differentiation in the ubiquitous Antarctic benthic invertebrate, *Orchomenella franklini*. Genetic differentiation was most pronounced across ~1400km between Casey and Davis, indicating that populations in these two regions are effectively isolated. At local scales, genetic differentiation was consistent with a stepping-stone model of dispersal and we conclude that individuals maintain gene flow over hundreds of metres, but that dispersal across larger distances occurs rarely. In addition, we found evidence of differential selection occurring between the Casey and Davis populations and suggest this represents localised adaptation in *O. franklini*. The lack of gene flow among populations and evidence of selection provide an insight into processes of speciation in Antarctic brooders, and should be considered in future management initiatives for the Antarctic benthos.

3.5.1. Contrasting levels of genetic diversity in *O. franklini* populations

While microsatellite variation in all *O. franklini* populations was considerable, genetic diversity was significantly lower at Casey than at Davis. Contrasting levels of genetic diversity among populations of high latitude species are often suggested to reflect signatures of historical glaciation (Marko 2004; Wilson *et al.* 2007; Baird *et al.* 2011), however this is unlikely to explain our data, as microsatellite variation should reflect more recent demographic processes (Selkoe & Toonen 2006). One potential explanation is differences in the spatial heterogeneity of Casey and Davis: it is generally accepted that greater environmental heterogeneity will maintain a higher level of genetic variation within species (reviewed in Hedrick 1986). While there is no published comparison of Casey and Davis environments, studies have generally emphasised the high heterogeneity of Davis benthic habitats, which include fjords and fjord mouths, open wave-exposed coast and significant quantities of wind blown sediment resulting from large ice-free areas (Everitt *et al.* 1980; O'Brien *et al.* In press). Preliminary measurements of sediment properties also indicate greater heterogeneity at Davis, with a range in mean sediment grain size range among Davis locations of approximately 516μm and a range among Casey locations of approximately 222μm; similarly, the total organic carbon content of sediments ranges up to 15% at Davis, and

only up to 10% at Casey (the authors, unpublished data). Different levels of iceberg scouring (see Demarchi *et al.* 2010) and interspecific competition (see Leese *et al.* 2010) have also been proposed to explain contrasting genetic diversity among Antarctic benthic invertebrate populations, providing further plausible explanations for the observed differences between Casey and Davis.

There was some evidence of an effect of local anthropogenic pollution on genetic diversity. Although allelic richness and heterozygosity measures appeared unaffected, the number of private alleles per population was lower in polluted locations. Private alleles are an important measure of genetic diversity (Kalinowski 2004), and a reduction in private alleles may occur through many mechanisms including population bottlenecks, selection against sensitive genotypes or even depressed mutation rates due to contaminants (Bickham *et al.* 2000). While elucidating the contribution of these processes to reduced genetic diversity in polluted *O. franklini* populations is beyond the scope of this study, the result indicates that further examination of the genetic effects of anthropogenic pollution on Antarctic benthos will be important. Interestingly, significantly lower genetic diversity has also been observed for contaminated populations of a closely related amphipod (*Orchomenella pinguis*) from the Arctic (Bach 2009), suggesting that amphipods may be useful bioindicators of anthropogenic induced genetic change in polar regions.

3.5.2. Restricted gene flow in *O. franklini*

Genetic differentiation among populations of *O. franklini* was greatest between the two major geographical regions Casey and Davis, which explained 69% of all microsatellite variation observed. Significant F_{ST} of 0.16, pairwise F_{ST} values of up to 0.2, and R_{ST} values up to nearly 0.5 indicate that the two regions are effectively isolated. Importantly, this significant genetic differentiation was still evident after removal of loci under selection, confirming that genetic drift due to restricted gene flow is important in driving this strong genetic subdivision between Casey and Davis. Estimated N_em of 1.4 provides further evidence that there is insufficient exchange of individuals between these regions to prevent them from diverging on independent evolutionary trajectories (Slatkin 1987; Lowe & Allendorf 2010).

Gene flow in *O. franklini* is also limited across relatively small spatial scales. Although there was no significant differentiation revealed between replicate sites within

locations, indicating that animals are panmictic over 100s of metres, we did find genetic differentiation among locations within regions (i.e. across distances of 1-30km). There was a clear pattern of isolation by distance within both Casey and Davis, indicating that migration occurs primarily between adjacent populations (Kimura & Weiss 1964). This is one of the first reports of population differentiation over such a small distance for an Antarctic benthic invertebrate. Limited gene flow over these scales is consistent with the brooding development in *O. franklini*, which predicts highly restricted capacity for dispersal. Indeed, similar findings have been reported for brooding taxa from temperate and tropical regions (e.g. Hess *et al.* 1988; Carvalho 1989; Wilson *et al.* 1997; Bastidas *et al.* 2002). Whilst some studies of brooding Antarctic invertebrates have also revealed strong intraspecific structure (Arango *et al.* 2010; Baird *et al.* 2011), other Antarctic brooders have shown evidence of gene flow over remarkably large distances, purported to reflect passive dispersal via the Antarctic Circumpolar Current (Mahon *et al.* 2008; Leese *et al.* 2010). Clearly, such a mode of dispersal does not occur in *O. franklini*, despite its wide distribution around the Antarctic coast (De Broyer & Jażdżewski 1993), as verified in this study by the absence of any evidence for cryptic species. Similar to conclusions drawn by Arango *et al.* (2010) for a circum-Antarctic brooding pycnogonid, we suggest that *O. franklini* has achieved its widespread distribution through historical colonisation, but that contemporary gene flow over large distances is severely limited. Such restricted gene flow is likely to promote allopatric speciation between populations and therefore supports the notion that limited dispersal has contributed to the high species diversity observed in Antarctic amphipods (De Broyer *et al.* 2003b) and brooding taxa in general (Cohen & Johnston 1987).

3.5.3. Evidence of local adaptation

We detected directional selection acting between Casey and Davis populations of *O. franklini* at locus *Orcfra4,* providing further evidence that these two regions are isolated and evolving independently. Although microsatellites are considered a neutral marker, they have increasingly been shown to reflect selection by genetic hitch-hiking (Nielsen *et al.* 2006; Larsson *et al.* 2007; Hemond & Wilbur 2011). Rather than rendering them uninformative, this provides valuable, biologically-relevant information on population structure (Vasemägi & Primmer 2005; André *et al.* 2011). Selection did not appear to occur between locations or sites within each region, suggesting there

exists a large-scale selection pressure, to which populations across entire regions are differentially adapted. This selection provides a likely explanation for the significant inbreeding observed at *Orcfra4* for most Davis populations, yet none from Casey. Additionally, loci *Orcfra5* and *Orcfra12* showed evidence of balancing selection, indicating that homogenizing selection pressures also act across both of the regions.

Localised adaptation of Antarctic benthic populations has barely been researched to date, as the stability of the environment has long fostered the view of a relatively homogenous fauna (Hedgpeth 1970; Dell 1972; Arnaud 1977; White 1984). Whilst this theory has since been dispelled by observations of distinctly heterogeneous species assemblages (Raguá-Gil *et al.* 2004; Gutt 2007), little genetic research has addressed the issue. Locally adapted populations may reduce the potential for a species to respond cohesively to broad-scale environmental change, as advantageous alleles will not have the opportunity to become widespread (Levins 1964; Slatkin 1987). Rather, local adaptation is likely to facilitate speciation, as populations subject to differential selection pressures become more genetically isolated over time (Ehrlich & Raven 1969; Rieseberg *et al.* 2004). For *O. franklini*, the potential for speciation between Casey and Davis populations will ultimately be determined by the interplay of both directional and balancing selective forces, along with continued genetic drift in the face of restricted gene flow.

3.5.4. Implications for conservation and future research

The geographical isolation of *O. franklini* populations has important implications for the future design of Antarctic MPAs. To maintain connectivity in this species and replenish any diminished populations outside reserve boundaries, a very close spacing of protected areas would be required. Of course, final management designs must incorporate such information from a wide variety of taxa, nevertheless, the high prevalence of brooding in Antarctic benthic species (Picken 1980; Knox 2007) suggests that maintaining connectivity between reserves will emerge as a key design challenge. Of further importance to Antarctic benthic management is the different levels of genetic diversity observed within *O. franklini* (e.g. between Casey and Davis populations). Conserving genetic diversity within species is crucial as it provides the raw material for adaptation to changing conditions, hence facilitating long-term persistence (Lande & Shannon 1996; Bowen 1999). Thus, if management efforts

inadvertently protect populations with lower genetic diversity, as has already been shown to occur in one established marine reserve (see Bell & Okamura 2005), the evolutionary potential of species may be compromised. Our study also provided preliminary evidence of a loss of genetic diversity in polluted populations, which may further increase their susceptibility to any ongoing stressors (Nevo *et al.* 1986; Guttman 1994). Such indications of anthropogenic induced genetic change require further attention in the Antarctic, where pollutants are highly localised (Lenihan *et al.* 1990), yet their effects on marine fauna are largely unknown (Chapman & Riddle 2005).

Clearly, intraspecific genetic structure is a field that warrants increased research in the Antarctic benthos. To date this has been hampered by the logistical difficulties of sampling such an extreme environment (Griffiths 2010), as well as by the common discovery of cryptic species, which drastically lowers intraspecific sample size. Our results highlight that future research should address intraspecific gene flow over several spatial scales, as mechanisms acting over one scale may not be apparent over another. Despite Antarctica's suite of remarkably stable environmental features and long-held views of a homogenous fauna, our study suggests that populations may be adapted to local selection pressures within the Antarctic benthic environment, and this may help explain the high rates of speciation in amphipods and other Antarctic brooders. The continued use of microsatellites and other highly variable molecular markers should further illuminate such microevolutionary patterns in the Antarctic benthos (Held & Leese 2007), although increased research on the underlying ecology of species will help interpret the patterns revealed, in particular the processes driving local adaptation. Ultimately, this will improve our understanding of Antarctic benthic species responses to environmental change, and how best to manage this unique environment.

Chapter 4. Population dynamics, abundance and distribution of the ubiquitous Antarctic benthic amphipod *Orchomenella franklini*

4.1. ABSTRACT

Comprehensive ecological information is still lacking for many of the dominant species of the Antarctic benthos, preventing an adequate understanding of their potential response to environmental change. One of the most ubiquitous members of the shallow Antarctic benthos is the amphipod *Orchomenella franklini*. The population dynamics of *O. franklini* was explored using measurements of sex and body length from over 6000 individuals, and patterns of abundance were examined and related to environmental parameters. Several life history traits were revealed for *O. franklini* that exemplify adaptations predicted for a polar environment. These include delayed reproduction, extended brood incubation, low fecundity, longevity and seasonal breeding linked to the summer phytoplankton bloom. There was also preliminary evidence of inter-annual and spatial fluctuations in population structure, potentially reflecting local environmental heterogeneity. *O. franklini* was found to reach astounding densities (> 65,000/m^2) and abundance was highly patchy, both spatially and on temporal scales. There was evidence of a relationship between the distribution of *O. franklini* and sediment properties, which was consistent with its trophic niche. The influence of both large scale and local environmental conditions on the ecology of *O. franklini* provides insight on the vulnerability of this species to environmental change.

4.2. INTRODUCTION

Knowledge of the population ecology of individual species is required to understand the potential effects of environmental change on marine ecosystems (Stenseth & Mysterud 2002; Harley *et al.* 2006). Studies on invertebrate species inhabiting the unique benthic environment of Antarctica have revealed several characteristic ecological traits, which indicate that they may be particularly vulnerable to environmental change (see: Clarke & Crame 1992; Peck 2005; Doney *et al.* 2012). These traits include life cycles tied closely to seasonal sea-ice dynamics and typically "K-adapted" life history strategies (see Pianka 1970) such as delayed maturity, slow growth and longevity (e.g. Arnaud 1977; Clarke 1979; Arntz *et al.* 1992; Brey & Clarke 1993). However, the general trends documented from the few species studied are unlikely to apply to all taxa and the early impetus of autecological work in the Antarctic has not been sustained (Arndt & Swadling 2006). Furthermore, an emphasis on the relative homogeneity of Antarctic conditions means there is little knowledge of local structuring of population ecology (Gutt 2007). This is especially so for the Amphipoda: despite being one of the most diverse and successful groups in the Antarctic benthos (see Brandt 1999; De Broyer *et al.* 2003b), we know relatively little about their population ecology (Arntz *et al.* 1992; Brandt 2000; Arndt & Beuchel 2006). Amphipods are ideal indicators for environmental assessment in polar regions (Duquesne *et al.* 2000; Bach 2009), hence understanding their ecology is important to provide the context against which environmental impacts can be assessed and interpreted. Moreover, the remarkable diversity and dominance of amphipods in the Antarctic (e.g. Jażdżewski *et al.* 1991; De Broyer & Jażdżewski 1996; Brandt 1999) makes them an ideal group in which to explore the ecological requirements for success in this environment.

The strong annual pulse of primary production in the Southern Ocean drives seasonal reproduction in a wide range of Antarctic benthic invertebrates (Clarke 1988; Pearse *et al.* 1991), yet this paradigm is far from being adequately tested for the Amphipoda. The few studies of reproduction in Antarctic amphipods reveal strategies ranging from a single brood released during the summer phytoplankton bloom (e.g. Sagar 1980; Rakusa-Suszczewski 1982), to a mid-winter brood release (e.g. Coleman 1989; Klages 1993), and even several successive broods annually (e.g. Bregazzi 1972). It has also been suggested that the majority of amphipods may be completely uncoupled

from the Antarctic seasonal cycle, as the lack of pelagic larvae together with a prevalence of predatory, scavenging and deposit-feeding modes absolves their need to rely on fresh phytoplankton (Arntz *et al.* 1992; Klages 1993). Other life history traits in Antarctic amphipods appear to be more consistent with general predictions for high-latitude strategies, i.e. relatively slow growth, delayed sexual maturity and long lifespans (Sainte-Marie 1991; Johnson *et al.* 2001). However, these observations are based on just a handful of the many benthic amphipod species recorded to date in Antarctica (Arntz *et al.* 1992). Exploring life history strategies in widespread and abundant species is important to understand adaptation to the unique prevailing conditions of the Antarctic environment.

An emphasis on the relative uniformity of many Antarctic benthic conditions (see Gutt 2007) has hindered any thorough examination of the influence of local environmental processes on population structure. Furthermore, existing autecological studies often lack adequate temporal or spatial coverage to explore intraspecific variation in population dynamics (Arntz *et al.* 1992; Griffiths 2010), reflecting the difficulties of sampling in polar regions (Arndt & Swadling 2006). Nonetheless, for some Antarctic amphipod species there is evidence that population size structure varies spatially within some Antarctic amphipod species (Sagar 1980; Slattery & Oliver 1986) and may reflect local ice dynamics. Isolated studies on other Antarctic benthic invertebrates also indicate that both population structure (e.g. Gutt *et al.* 1992) and fecundity (e.g. Wägele 1987b) may vary according to habitat, although there has been minimal investigation of the actual environmental parameters responsible. There is even less information on temporal fluctuations in Antarctic benthic population ecology. However, recent studies in some Antarctic benthic invertebrates are beginning to highlight inter-annual variability as an important factor to consider in understanding local population abundance and reproductive output (e.g. Chiantore *et al.* 2002; Grange *et al.* 2007; Thrush & Cummings 2011). In summary, there is growing evidence to suggest that local-scale processes may be just as influential in structuring Antarctic benthic populations as the broader, prevailing Antarctic conditions, which remain the focus of research to date.

The effect of physical disturbance by ice on the abundance and distribution of Antarctic benthic species has been well documented (e.g. Gutt 2001; Gerdes *et al.* 2003; Brown *et al.* 2004). However, much less is known about the influence of any other environmental factors on spatial variation in species' abundances (Gutt 2007). Particular

uncertainty surrounds the relationship between sediment attributes and Antarctic benthic distribution. The current theory is that most Antarctic benthic invertebrates are largely opportunistic in substrate choice and thus are not significantly structured by sediment properties (Gutt 2007). For amphipods the few observations available only add confusion, for example Jażdżewski *et al.* (1991) argued that different sediment characteristics drove different amphipod species assemblages, whereas Tucker & Burton (1988) suggested that amphipod distributions were unaffected by substrate. Elucidating these patterns is important given that marine sediments will be affected by ongoing localised anthropogenic pollution in the Antarctic, as well as broad-scale climate change.

The amphipod *Orchomenella franklini* is one of the dominant members of the Antarctic nearshore benthos (Tucker & Burton 1988; Stark 2000; Knox 2007), where it plays a significant trophic role as prey for notothenioid fish (La Mesa *et al.* 2007). *O. franklini* is ubiquitous around the Antarctic coast (Lowry & Bullock 1976; De Broyer *et al.* 2007) and is often highly abundant in polluted locations (Stark 2000; Stark *et al.* 2004), making it a particularly interesting subject in which to explore questions relating to its ecological success. However, little is known about its life history: Tucker (1988) provided preliminary evidence that breeding in *O. franklini* is synchronised with the summer peak in phytoplankton, yet Stark (2000) suggested that year-round breeding aided its numerical dominance. While *O. franklini* is known to have a patchy distribution (Stark 2000), its abundance has been claimed to be unaffected by substrate (Tucker 1988). We explored the population dynamics, abundance and distribution of *O. franklini* in order to address the following questions:

- does the life history of this amphipod reflect typical "K-adapted" and seasonally timed strategies predicted for success in high latitude regions such as the Antarctic?
- does the ubiquity of *O. franklini* and the relative uniformity of many broad-scale Antarctic conditions result in homogenous population dynamics and distribution (both spatially and temporally), or is there evidence of locally-structured populations?
- does the distribution of this amphipod support the theory that sediment properties play a negligible role in structuring benthic invertebrate distributions in Antarctica?

By resolving these questions we also aimed to shed light on the potential sensitivity of this dominant Antarctic benthic species to environmental change.

4.3. METHODS

All samples used for this study (except for those collected at Davis station: see Section 4.3.2.1) were archival samples provided by the Australian Antarctic Division. This opportunistic approach enabled us to maximise the temporal and spatial extent of data to provide a more comprehensive overview of ecological dynamics in *Orchomenella franklini*.

4.3.1. Population dynamics

4.3.1.1. Samples

Samples were collected at Casey station in East Antarctica (66º17´S, 110º32´E; Figure 4.1) during several sampling periods from October 1997 to December 2006 (see Table 4.1). Samples were taken by SCUBA divers during the spring and summer months, with cylindrical PVC cores of 10cm diameter pushed 10cm deep into the sediment. Samples from the winter months were taken by a van-veen grab sampler deployed through holes drilled in the sea-ice. Cores and grab samples were either preserved in 5-10% formalin with Biebrich scarlet stain, or frozen (in which case they were stained with Biebrich scarlet upon thawing). Samples from seven different locations covering ~10km of coastline (Figure 4.1, Table 4.1) were used, in order to investigate the spatial variability of population dynamics. Locations were given a classification reflecting their average onset and duration of summer sea-ice breakout ('low', 'intermediate' or 'high' from minimal to maximal duration of breakout; Table 4.1), based on personal observations during field seasons, and light meter measurements (Martin J. Riddle, unpublished data). Due to logistical constraints (e.g. weather and ice conditions), sampling was not achieved for all locations each year, nor was it achieved during the autumn months (see Table 4.1).

Cores and grab samples were sieved on a 0.5mm mesh, which is known to retain the smallest *Orchomenella franklini* specimens (Stark *et al.* 2003a). Retained benthos was sorted under a dissecting microscope and all *O. franklini* individuals were removed, counted, measured and sexed. If there were more than 100 specimens in a core/grab sample, a subsample of 100 individuals were randomly selected to be measured and sexed.

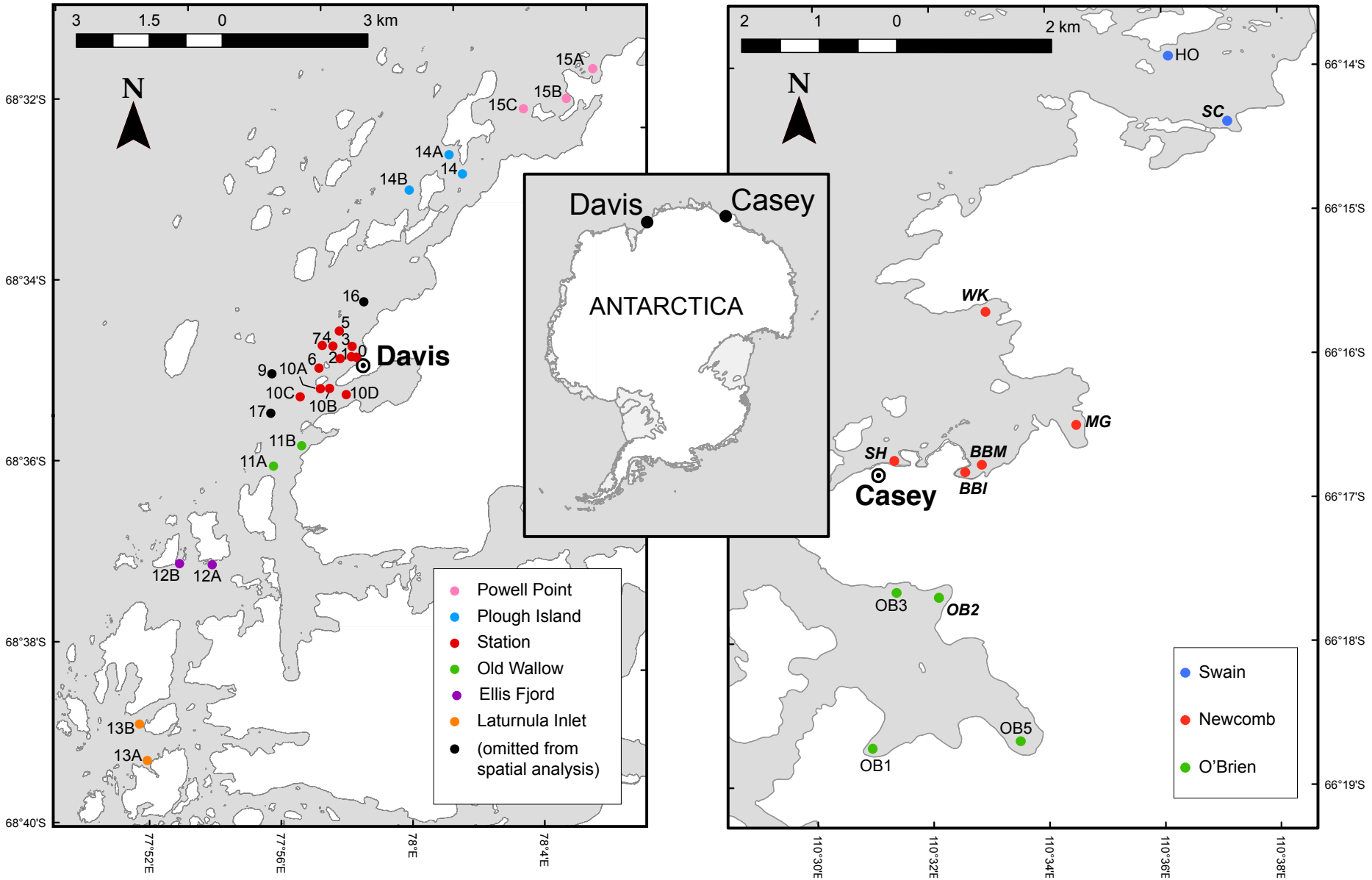

Figure 4.1: Locations at Casey and Davis stations from which *O. franklini* were collected, with inset showing the position of Casey and Davis on the Antarctic coast. The seven locations at Casey from which *O. franklini* samples were used to explore population dynamics (Section 4.3.1.) are italicised. All locations are coloured by the broad area in which they were placed to analyse spatial patterns of abundance (Section 4.3.2.).

Table 4.1: *O. franklini* population dynamic sample sizes from Casey for each location and month, with sampling year given in parentheses. Total monthly sample sizes used for metapopulation reproduction and growth analyses (i.e. pooled over sampling years and locations) are also shown.

		Sample size (*sampling year*)							
Degree of		**Winter**			**Spring**			**Summer**	
sea-ice breakout	Location	June	July	Aug	Sept	Oct	Nov	Dec	Jan
low	Brown Bay Inner ('BBI')	150 (*'98*)	49 (*'98*)		61 (*'98*)	747 (*'97*)	200 (*'05*), 125 (*'06*)		
	Brown Bay Middle ('BBM')						132 (*'06*)	200 (*'05*)	998 (*'03*)
	McGrady Cove ('MG')						114 (*'05*), 41 (*'06*)		
	Stevenson Cove ('SC')							106 (*'06*)	
intermediate	O'Brien Bay 2 ('OB2')			25 (*'98*)			27 (*'97*)		
	Shannon Bay ('SH')				319 (*'98*)		65 (*'06*)	986 (*'97*)	845 (*'03*)
high	Wilkes ('WK')							264 (*'97*), 46 (*'05*)	523 (*'99*), 362 (*'03*)
Month totals (years & locations pooled):		150	49	25	380	747	707	1602	2728

4.3.1.2. Proxies for length measurements

To measure *O. franklini* specimens, first an appropriate estimator for total body length was sought. The natural curvature of the body of amphipods, as well as the "telescoping" of segments underneath one another makes measuring total length imprecise (Quigley & Lang 1989; France 1993; Chapelle 1995). Also, preliminary observations showed that the telson, typically included in total length measurements (Figure 4.2), was often damaged or completely absent, most likely a consequence of freezing, thawing and/or sieving processes. The dorsal length of both the head and the first pereonite (Figure 4.2) were tested for their correlation to total body length in *O. franklini*, as both have been found to be successful proxies for total length in other amphipod species (Strong 1972; France 1993; Wilhelm & Lasenby 1998; Arndt & Beuchel 2006). Fifty-five specimens (25 male, 30 female) with intact telsons and covering a wide size range were selected from several locations, and measured three times for each length parameter. A Leica Z6 APO A microscope with Image Analysis software was used to determine total length while the animal remained in its natural position of curvature. The correlation of total length with the dorsal length of both the head and the first pereonite was tested using linear regression performed in R 2.12.2 (R Core Development Team 2011). Both proxies were highly correlated to total length (Figure S4.1[1]). The first pereonite displayed the strongest correlation ($R^2 = 0.98$ $p < 0.001$; Figure S4.1) and was therefore used subsequently to estimate total length, through the equation derived from regression:

$$\textit{total length} = (\textit{dorsal length first pereonite} \times 15.05) + 0.38.$$

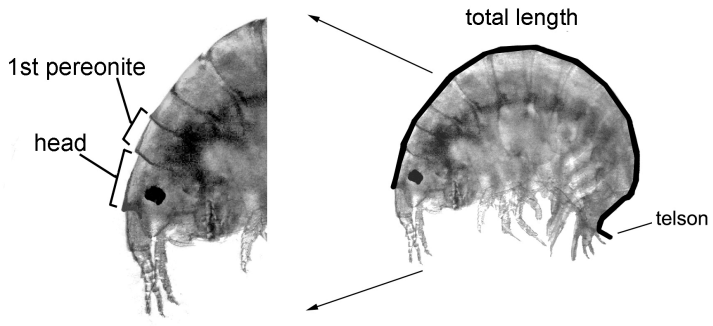

Figure 4.2: Photograph of an adult *O. franklini* specimen showing the length parameters tested for their correlation in this study.

[1] All supplementary figures and tables for this chapter are provided in Appendix III

4.3.1.3. Size cohorts and growth

The length of individuals was measured in order to elucidate size cohorts, which would provide insight on the growth and life cycle of *O. franklini*. The dorsal length of the first pereonite was measured three times in each animal with a 0.1mm ocular micrometer under a Wild Heerbrugg M5 dissecting microscope at 50× magnification (providing 0.02mm accuracy). The average of the three measurements was then converted to total length, using the formula derived in Section 4.3.1.2.

A normal mixture model was fitted to length data so that discrete size cohorts could be identified and the mean length estimated for each, in order to model growth. Normal mixture modeling was performed with the MClust package for R (Fraley & Raftery 2002), which fits a mixture of normal distributions by the EM algorithm, selecting the best model by the Bayesian Information Criterion (BIC). Length measurements from November, December and January samples were used, as these months provided large sample sizes and incorporated data from at least three locations. All females that had broods or had just released a brood were removed from the dataset before analysing cohorts. There is strong evidence that female amphipods exhibit reduced growth during and after brood incubation due to an altered allocation of resources (Sainte-Marie *et al.* 1990; Poltermann 2000) and will therefore not follow the growth pattern exhibited by juveniles, males and immature females (Takeuchi & Hirano 1991). Hence, their removal from the analysis ensured they would not influence normal curves and growth models produced from the data. Analysing male and female data separately was not feasible as this would require removal of all juveniles from the dataset, thus drastically reducing sample size. Length data were log-transformed to provide improved model fits (as indicated by BIC values) and the variance of each normal component was allowed to vary.

Normally-distributed length cohorts in crustaceans can indicate either moult stages or age classes (see Schmidt *et al.* 2002). For *O. franklini,* cohorts identified by normal mixture modeling were assumed to represent year classes, as our data strongly indicated that this species releases offspring during a single defined period each year (see Section 4.4.1.2.), as do the majority of polar amphipods (Sagar 1980; Sainte-Marie 1991; Poltermann 2000; Węsławski & Legeżyńska 2002; Arndt & Beuchel 2006). Thus, the mean (log-transformed) length values extracted from each cohort could be used to model growth as a function of age. The smallest cohort from the November dataset was

nominated as age = 0, since this cohort represents newly released offspring (see Section 4.4.1.2.). Most amphipods exhibit either linear or exponential growth rather than the more commonly used asymptotic models (Highsmith & Coyle 1991) so we fitted a linear model to the log-transformed length means for each cohort (*i.e.* exponential growth), as well as to the anti-log of these values (*i.e.* linear growth). Models were fitted in the program R.

4.3.1.4. Sex, maturity and reproduction

The sex and maturity of animals and the reproductive status of females were determined, so that population structure and life history strategy could be explored in *O. franklini*. Individuals were classed as males (M) if penile papillae and/or antennal calceoli were observed, females (F) if oostegites or oostegite buds were observed, and brooding females (B) if the brood pouch contained one or more eggs or juveniles. A fourth category, 'recent brooders' (RB), discriminated females that had recently released offspring. These animals are characterised by an expanded brood pouch and enlarged oostegites but no eggs or juveniles present, although some debris often remains (see Hill 1988; France 1993; Thurston *et al.* 2002; Arndt & Beuchel 2006). Reproductive organs were not discernable in animals < 4.50mm total length, so these were classed as juveniles (J); a common approach in amphipod research (Sagar 1976; Takeuchi & Hirano 1991; France 1993; Dale *et al.* 2006). Sex ratios were tested for a significant departure from equilibrium (1:1) using a chi-square distribution.

To assess fecundity and examine the correlation between brood size and female size (a predicted ecological trait in amphipods), eggs within the brood pouch of gravid females were counted when possible (specimen integrity was required for a subsequent study). This was achieved for 78 individuals (> 25% of all gravid females), whose total length covered a range that encompassed > 95% of all gravid females. The total length of a female and the number of eggs within her brood pouch were tested for a correlation using linear regression, performed in R.

4.3.1.5. Metapopulation life cycle

To discern the overall life cycle of *O. franklini*, we examined length-frequencies combined with population structure data (i.e. sex and reproductive status) for each sampling month separately, as preliminary evidence suggested that reproduction in *O.*

franklini would be seasonal (Tucker 1988). However, data for each month was pooled across different sampling years and locations to maximise sample size (as in Arndt & Beuchel 2006) and to avoid misinterpreting metapopulation dynamics on the basis of any single (potentially unique) population.

4.3.1.6. Local population structure

To determine if population dynamics in *O. franklini* are spatially and temporally homogeneous, we also compared population structure between different sampling years and locations. Due to seasonal reproduction resulting in distinct monthly differences in population structure, comparisons between different locations and years were only considered within the same sampling month. This reduced sample size such that it was unfeasible to explore differences between discrete size cohorts. Rather, we made qualitative observations on the differences in overall population composition.

4.3.2. Abundance and distribution

4.3.2.1. Abundance data

In order to make quantitative comparisons of the abundance of *O. franklini* in both space and time, abundance estimates were derived from core samples, which are a uniform size and shape (10cm diameter × 10cm deep). Cores were collected at both Casey station and Davis station in East Antarctica (Figure 4.1) over a range of years (Table 4.2), as described in Stark *et al.* (2003a). Within both of these regions, sampling was spatially hierarchically nested. Four replicate cores were taken within a plot (covering $\leq 10m^2$ area), and at least two plots (spaced between 10m and 100m apart) were sampled within a location. Locations themselves were between 100m and 2500m apart. For the purposes of this study, locations have been grouped into areas, which are > 2km apart and generally represent distinct bays or inlets, separated by terrestrial features (Figure 4.1, Table 4.2).

To maximise efficiency, abundance was estimated by sieving cores on a 1mm mesh size (see Thompson *et al.* 2003). However, 132 cores from a range of locations were sieved at both 0.5mm and 1mm (see Table 4.2) to validate the use of 1mm-derived abundance estimates as a predictor for total (i.e. 0.5mm-derived) abundance. This was tested using linear regression, performed in R.

Table 4.2: Samples for abundance data from Casey and Davis, detailing the geographic area and location, the number of plots and the month and year they were sampled. A minimum of 4 replicate cores were sampled within each plot. Plots shown in bold had a subset of cores sieved on both a 1mm and 0.5mm mesh (all others were sieved to 1mm only). Plots with corresponding samples available for sediment data are also indicated.

CASEY				DAVIS			
Area	Location	N° plots (date sampled)	Sediment data	Area	Location	N° plots (date sampled)	Sediment data
Newcombe	Brown Bay Inner (BBI)	**4 (Oct '97)**		Wharf	0	**2 (Mar '10)**	Y
		4 (Jan '03)			1	**2 (Feb '10)**	Y
		4 (Nov '05)			2	**2 (Feb '10)**	Y
		4 (Nov '06)	Y		3	**2 (Feb '10)**	Y
	Brown Bay Middle (BBM)	4 (Oct '97)			4	**2 (Feb '10)**	Y
		4 (Dec '97)			5	**2 (Feb '10)**	Y
		6 (Dec '98)			6	**2 (Mar '10)**	Y
		4 (Jan '03)			7	**2 (Mar '10)**	Y
		4 (Jan '04)			10A	**2 (Feb '10)**	Y
		4 (Dec '05)	Y		10B	**2 (Mar '10)**	Y
		4 (Nov '06)	Y		10C	**2 (Mar '10)**	Y
	McGrady Cove (MG)	4 (Jan '03)			10D	**2 (Mar '10)**	Y
		4 (Nov '05)	Y				
	Shannon Bay (SH)	**4 (Dec '97)**		Old Wallow	11A	**2 (Feb '10)**	Y
		4 (Jan '03)			11B	**2 (Feb '10)**	Y
		4 (Nov '06)	Y				
	Wilkes (WK)	4 (Dec '97)		Ellis Fjord	12A	**2 (Feb '10)**	Y
		4 (Jan '99)			12B	**2 (Feb '10)**	Y
		4 (Jan '03)					
		4 (Jan '04)		Laturnula Inlet	13A	**2 (Feb '10)**	Y
		4 (Dec '05)	Y		13B	**2 (Feb '10)**	Y
O'Brien	O'Brien Bay 1 (OB1)	**4 (Nov '97)**		Plough Island	14	**2 (Feb '10)**	Y
		4 (Jan '03)			14A	**2 (Feb '10)**	Y
		4 (Dec '05)	Y		14B	**2 (Feb '10)**	Y
	O'Brien Bay 2 (OB2)	**4 (Nov '97)**					
		4 (Nov '98)		Powell Point	15A	**2 (Mar '10)**	Y
		4 (Jan '03)			15B	**2 (Mar '10)**	Y
		4 (Nov '06)	Y		15C	**2 (Mar '10)**	Y
	O'Brien Bay 3 (OB3)	4 (Nov '97)					
		4 (Feb '03)		(omitted from spatial analysis)	9	**2 (Mar '10)**	Y
	O'Brien Bay 5 (OB5)	**4 (Dec '05)**	Y		16	**2 (Feb '10)**	Y
					17	**2 (Mar '10)**	Y
Swain	Honkala (HO)	**4 (Dec '06)**	Y				
	Stevenson Cove (SC)	**4 (Dec '06)**	Y				

4.3.2.2. Spatial and temporal variability in abundance

To explore spatial and temporal variation in the abundance of *O. franklini* we used PERMANOVA (Anderson 2001; McArdle & Anderson 2001), a permutation based equivalent to analysis of variance (ANOVA), which is more flexible to departures from assumptions of normality and for handling unbalanced data sets. This was performed with the PERMANOVA+ package for PRIMER 6 software (Anderson *et al.* 2008). For all analyses a log(x+1) transformation was applied to abundance data to address heterogeneity of variance and 10,000 permutations of the data were performed to assess significance.

For analysis of spatial variation we restricted tests to samples taken within a 30-day period. This was to remove any confounding effect of sampling year or month (see Section 4.4.2.2.). This resulted in three tests for spatial variation at Casey (for the sampling periods January 8th–February 8th 2003, November 18th–December 16th 2005, and November 8th–December 7th 2006) and one test at Davis (for the period February 12th–March 12th 2010). A 3-factor nested PERMANOVA, with area an orthogonal factor, location nested within area, and plot nested within location was used to test the hypothesis that abundance was homogenous across all spatial scales. All three factors were considered random because we were interested in general spatial variability, rather than patterns specific to the areas sampled (see Underwood 1997). If analyses yielded negative variance components, these were set to zero, the respective factor was removed from the analysis and estimates were recalculated, as recommended by Fletcher & Underwood (2002). Variance components were then converted to percentage total variance, to compare the relative magnitude of variation at each spatial scale (Graham & Edwards 2001).

We also tested whether abundance was homogenous across temporal scales. Inter-annual variation in abundance was determined by single factor PERMANOVA. To isolate the effect of year from any confounding spatial and monthly effects, inter-annual comparisons were restricted to data from the same location and sampling month. Single factor PERMANOVA was also used to test for intra-annual variation, i.e. an effect of month. To isolate the effect of month from any confounding spatial and inter-annual variability, we only had sufficient data to compare October and December of 1997 within one location at Casey.

4.3.2.3. Environmental data

To explore environmental effects on the distribution of *O. franklini*, data on various sediment parameters (Table S4.1) were generated from sediment cores. Sediment parameters reflecting both natural properties (e.g. grain sizes) and anthropogenic pollutants (e.g. heavy metals) were examined, as some of the locations investigated were contaminated (see Snape *et al.* 2001; Stark *et al.* 2003a; Stark *et al.* 2011). Cores for sediment analysis (5cm diameter x 10cm deep) were sampled with the same spatial design as cores used for abundance estimates, but with two cores taken per plot (and only for a subset of the sampling years at Casey, see Table 4.2). All equipment associated with the sampling and processing of sediment cores was washed prior to use in 10% nitric acid (HNO_3).

Total organic carbon (TOC) analysis was carried out on a 2g homogenised wet sub-sample, which was weighed into a pre-combusted crucible and dried at 105°C. The dried sample was reweighed, placed in a muffle furnace for 4 hours at 550°C and total carbon was calculated as mass loss on ignition.

For grain size estimation (parameters summarised in Table S4.1), the outer 5mm edge of the core was removed with a scalpel blade (to eliminate edge bias and coring effects) and the remainder placed in a clean dry beaker, weighed and placed in an oven at 45°C to dry. Once dry, the sample was reweighed and sieved through a 2mm mesh. The < 2mm fraction and the > 2mm fraction were then weighed separately. A 5g sample of the < 2mm fraction was taken for grain size analysis, carried out using the Mastersizer 2000 Particle Size Analyser at the Department of Physical Geography, Macquarie University, Sydney.

Analysis of bioavailable metals and other trace elements in sediments (Table S4.1) was carried out on < 2mm fractions, as this provides a more realistic estimate of metals in the sediment than the < 63µm fraction (a common alternative) or whole sediment (Loring 1991). A weak acid extraction was performed on a 3g sub-sample of homogenised wet sediment, mixed with 30mL of 1M HCl in a polypropylene centrifuge tube and tumbled for 4 hours at room temperature. The mixture was then centrifuged for 15 minutes at 12,000rpm and the supernatant filtered through a 0.45µm membrane cartridge filter (Minisart, Sartorius AG). The filtered supernatant was stored at 4°C before analysis by ICP-MS at Central Science Laboratories, University of Tasmania.

4.3.2.4. Relationship between sediment properties and abundance

To determine whether the spatial variability of *O. franklini* abundance at Casey and Davis (see Section 4.4.2.2.) reflected environmental differences, we tested for spatial variability in sediment properties. Data for all measured sediment parameters were combined and Analysis of Similarity (ANOSIM) was used to test for differences in the sediments from each geographic area and location. ANOSIM was based on Euclidean distance similarity matrices and was performed with PRIMER 6 software.

We also examined the relationship between sediment parameters and abundance data, to determine the influence of sediment on the distribution of *O. franklini.* Principal component analysis (PCA) was used to represent the variation among samples based on sediment variables, as this technique is well suited to multivariate analysis of environmental data (Clarke & Warwick 2001) and is able to account for co-correlation of variables. Each environmental PCA was based on sediment cores averaged over the sampling plot (n = 2) and ordinations of the relationships among samples were plotted on the first two principal components. Points representing each sample were then scaled to represent the mean abundance of *O. franklini* in that particular plot (n = 4), so that patterns of abundance could be related to sediment variation. Furthermore, generalised additive modeling (GAM) was used to model *O. franklini* abundance as a smooth function of the first two principal components, which was overlayed as contours on PCA plots. While a Poisson distribution is the standard model used for count data (as it will not result in negative predictions), abundance data for *O. franklini* was extremely overdispersed. Therefore it was more appropriate to homogenise variance with a square-root transformation and fit a normal distribution. All PCAs were carried out in R and GAM was performed using the mgcv package for R (Wood 2006).

4.4. RESULTS

4.4.1. Population dynamics

4.4.1.1. Size and growth

A total of 6388 *Orchomenella franklini* specimens were measured. The smallest free-living juvenile was estimated at 2.50mm total length, the largest male 7.51mm and the largest female 9.02mm. Three distinct cohorts within log-transformed length data were identified in November, December and January by normal mixture modeling (Figure 4.3). These correspond to cohorts C0, C1 and C2 in the life cycle length-frequency histograms (see Section 4.4.1.2), since breeding females (C3) had been removed for normal mixture modeling. Incorporating the monthly growth of individual cohorts from November to January, as well as the yearly growth between cohorts, a strong linear relationship between $\log_e$(length) and age was identified:

$$\log_e(\textit{total length}) = 0.03\textit{age} + 1.03$$

($R^2 = 0.99$, $p < 0.001$; Figure 4.4). Thus there was strong evidence that the total body length of *O. franklini* increases exponentially with age (at least until females begin to breed). The estimated growth model (given by the antilog of the regression equation for log-transformed cohort data) for non-breeding *O. franklini* is therefore:

$$\textit{total length} = e^{0.03\textit{age}+1.03}$$

with total length in mm and age in months.

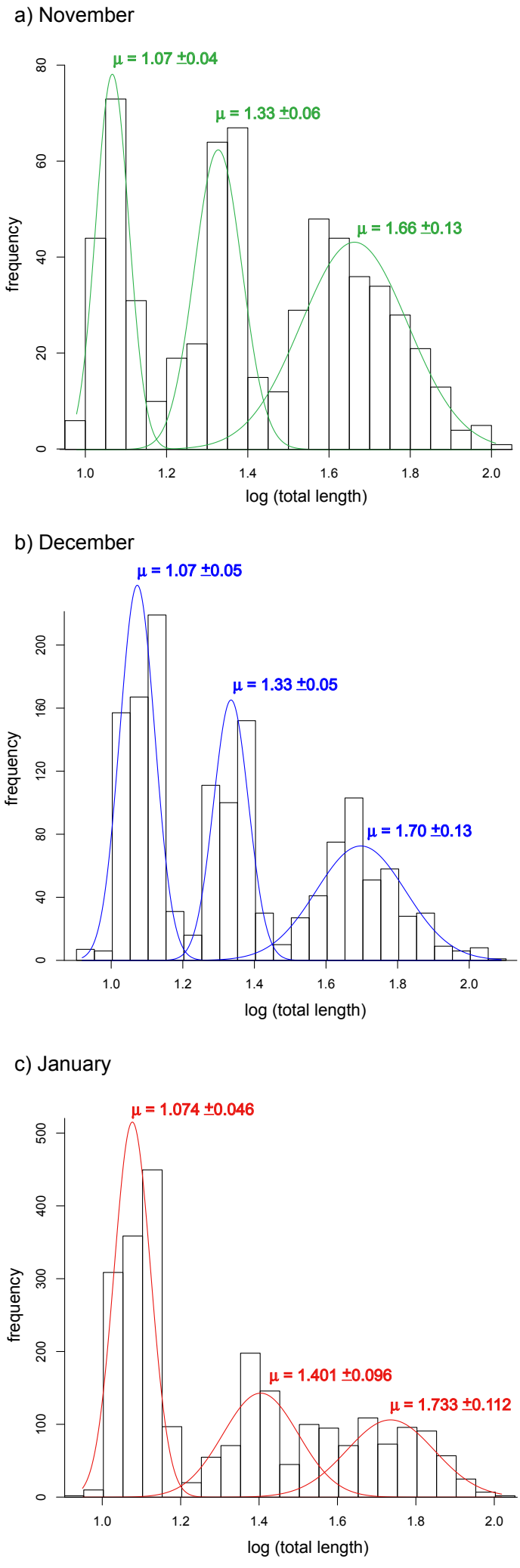

Figure 4.3: Log-transformed length-frequency histograms for *O. franklini* sampled in November (a), December (b), and January (c) (n = 626, 1461, and 2487 respectively; breeding females removed). Normally-distributed cohorts identified by the MClust mixture modelling package are shown in green (a), blue (b), or red (c), with corresponding mean values (μ) labelled above each cohort.

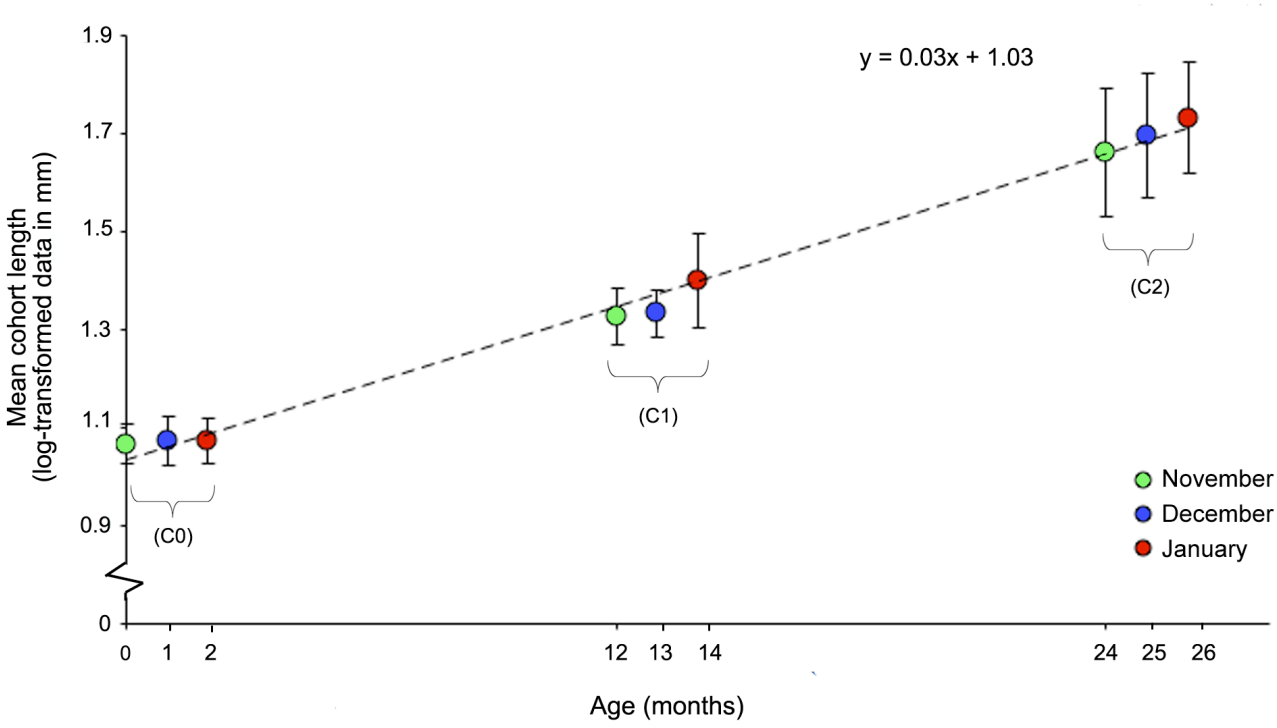

Figure 4.4: Growth model for *O. franklini* as determined by linear regression of identified length cohort means (see Figure 4.3), assuming cohorts represent year classes. Error bars represent the standard deviation for each estimated cohort mean.

4.4.1.2. Life history

Females were significantly more abundant than males across the entire sample of 6388 individuals (F/M = 1.11, $\chi^2 = 6.40$, $p = 0.01$). The number of eggs contained within a brood pouch ranged from one to seven and there was a significant correlation between the total length of a female and the size of her brood ($R^2 = 0.22$, $p < 0.001$). However, gravid amphipods are known to expel eggs due to the stress of capture (Sainte-Marie *et al.* 1990; Klages 1993), therefore these are considered minimum brood sizes.

O. franklini appears to have a single reproductive event per year, with brood release occurring over late spring/early summer. Samples taken from June to September (winter to spring) all contained brooding females (B) (Figure 4.5). Released broods (RB) were first found in October (late spring). Evidence of released broods continued

throughout early summer and by January very few females still retained broods (Figure 4.5). The brood incubation period for *O. franklini* is therefore at least four months long, although without data from February to May we cannot dismiss the possibility that it may be as long as eight months, should oviposition occur earlier than June. The proportion of juveniles in the population was greatest in January (67% of the population; Figure 4.5), coinciding with the end of the brood release period. During the winter months, the proportion of juveniles dropped to half this value (although they still comprised a third of the population: Figure 4.5).

Length-frequencies combined with sex and reproductive data (Figure 4.6) indicate that *O. franklini* has an approximately three-year life cycle. From June to September three size cohorts – or year classes – are evident. The first cohort (C1) contained only juveniles, the second (C2) contained adult males and females, and the third cohort (C3) contained almost entirely brooding females. In October the appearance of a new, smaller juvenile cohort (C0) was observed (i.e. newly released offspring), which remained through to at least January (with no samples measured after this date). Summarising the life cycle: offspring (C0) are released from brood pouches in late spring/summer, grow for one year into larger juveniles (C1), mature over their second year into (discernable) males and females (C2) and reproduce in their third year, resulting in brooding females (C3). In a few instances brooding females could be observed in cohort C2, indicating that reproduction may occasionally occur at a smaller size, presumably during the second year of life. While there was no evidence of a size cohort beyond C3, we cannot unequivocally state whether females die after releasing a brood, or simply cease to grow. However, we consider mortality likely after a single reproductive event, based on the majority of Antarctic benthic amphipods investigated (Sagar 1980; Arntz *et al.* 1992; Klages 1993). We also observed that females with evidence of a released brood were often in a poor physical state. Thus we propose that female *O. franklini* live to three years of age. Similarly, males most likely die after reproduction (there was no male cohort beyond C2), suggesting *O. franklini* males live for between two and three years.

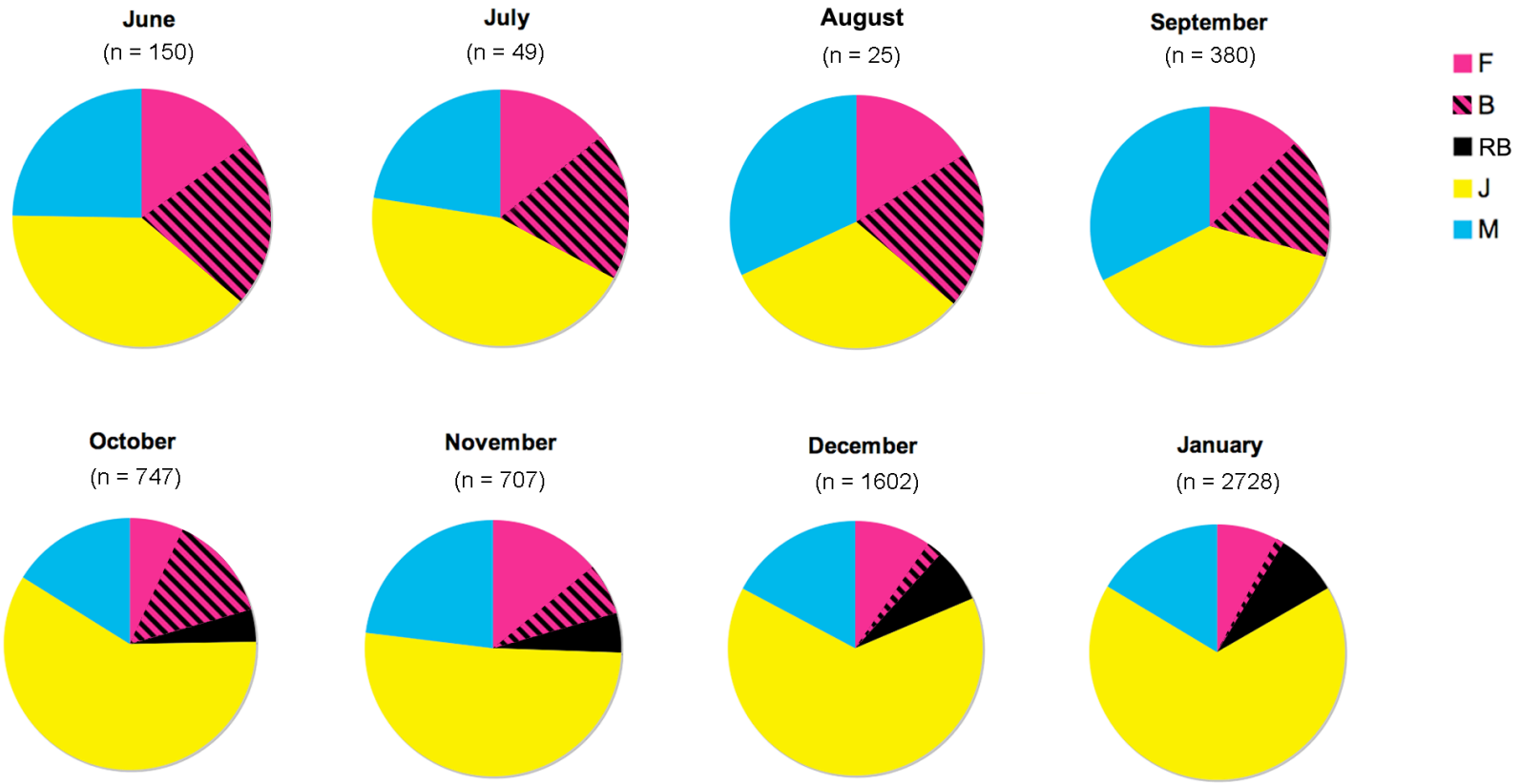

Figure 4.5: Monthly metapopulation composition of juveniles (J), adult male (M), adult female (F), brooding female (B) and female *O. franklini* that have recently released broods (RB). Sampling months extend from winter (June) through to summer (January).

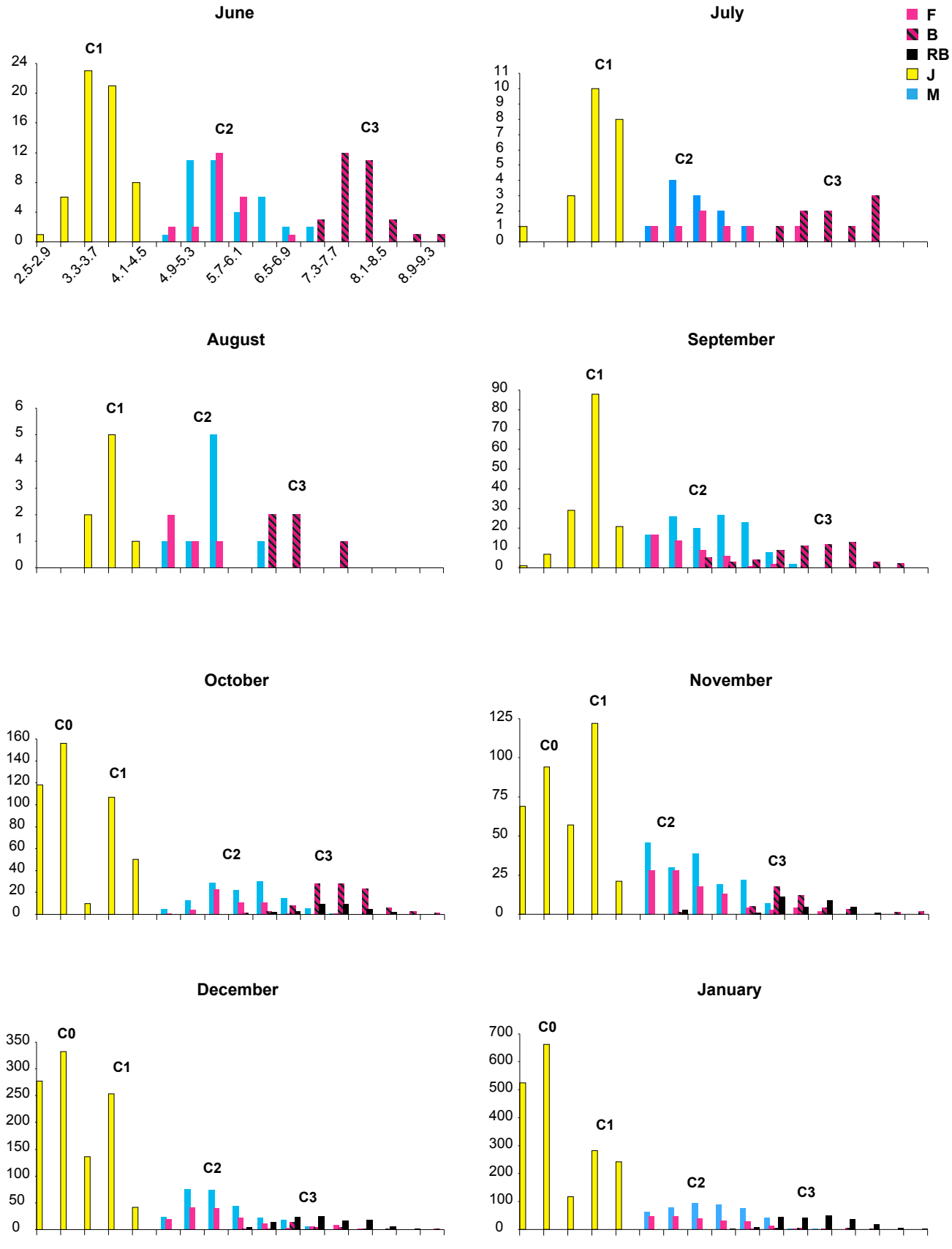

Figure 4.6: Monthly length-frequency histograms for *O. franklini* juveniles (J), adult males (M), adult females (F), brooding females (B), and females that have recently released broods (RB). The x-axis represents 0.4mm size classes (ranging from 2.5mm to 9.3mm total length, as shown for June), and the y-axis represents the number of individuals in each size class. Size cohorts are C0 (newly released juveniles), C1 (second-year juveniles), C2 (matured males and females), and C3 (almost entirely breeding females). Note that y-axis scales differ for each month.

4.4.1.3. Spatial and temporal variability of population structure

There was some evidence that population structure varied among years at a location (Figure 4.7), and also among locations within years (Figure 4.8). For example, in November at McGrady Cove, juveniles comprised 68% of the population in 2005 but only 32% in 2005 (Figure 4.7). Similarly in December 2005, 50% of the population at Brown Bay Middle were juveniles compared with > 90% at Wilkes (Figure 4.8). A higher proportion of juveniles might reflect a greater proportion of the season's breeding females having released their brood (i.e. RB / RB+B). Indeed, this appeared to be the case for three of the four inter-annual comparisons made (Figure 4.7), as it did for the majority of location comparisons (Figure 4.8). However we emphasise that these observations should be regarded cautiously due to low sample size. Although sex ratios also appeared to differ between both sampling years and locations (Figures 4.7 and 4.8), none departed significantly from equilibrium ($p > 0.05$ for all chi-square tests).

Variation in population structure among locations may be related to sea-ice cover. In three of the four location comparisons, there was an increase in both the proportion of broods released and the juvenile component of populations as the degree of sea-ice breakout progressed from low to high (Figure 4.8b).

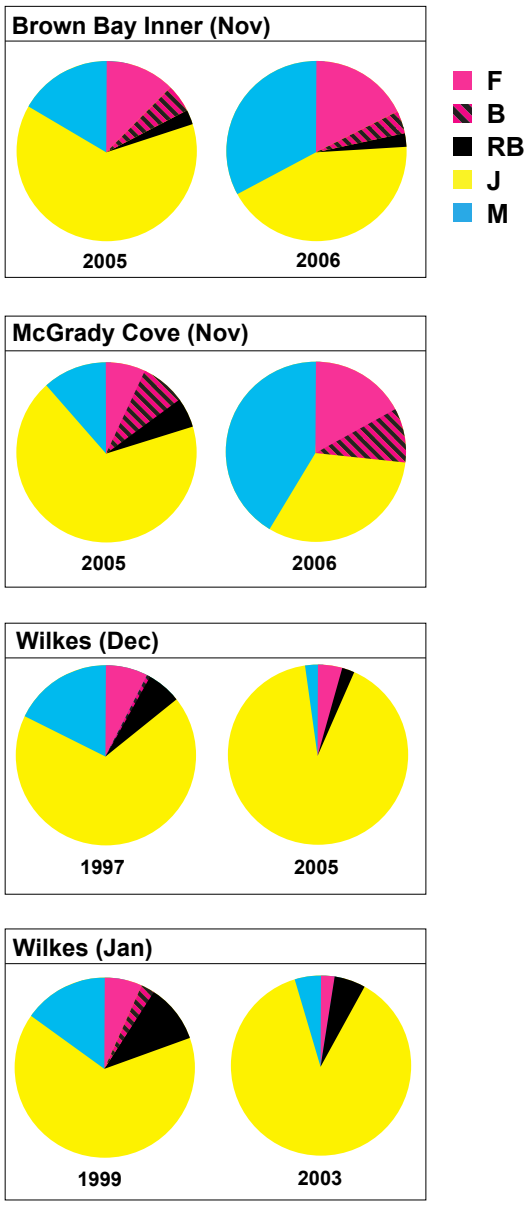

Figure 4.7: Inter-annual differences in population composition of *O. franklini* juveniles (J), adult males (M), adult females (F), brooding females (B) and females that have recently released broods (RB). Comparisons are restricted to data from the same month and location.

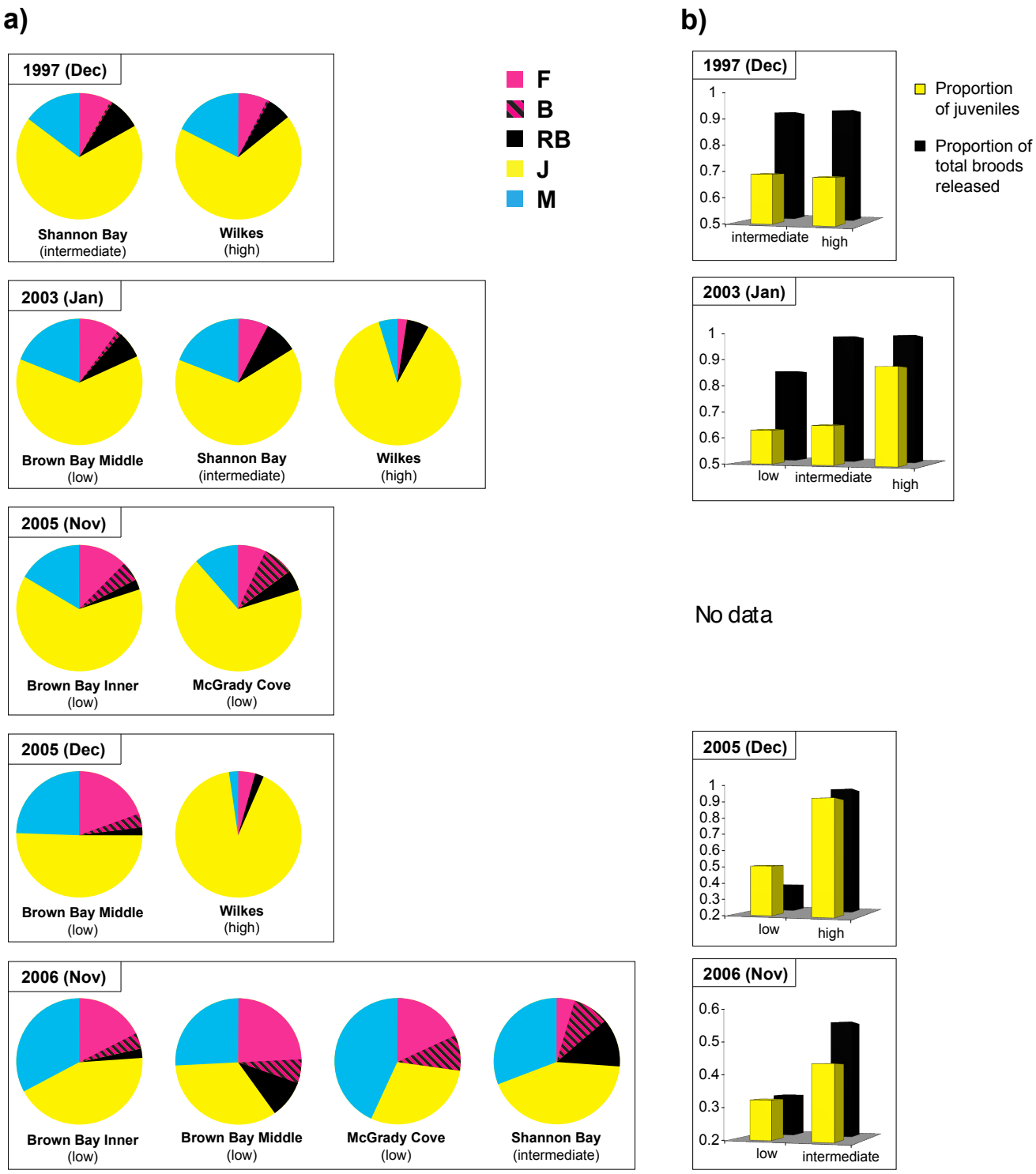

Figure 4.8: a) Location differences in population composition of *O. franklini* juveniles (J), adult males (M), adult females (F), brooding females (B) and females that have recently released broods (RB); b) comparison of the proportion of juveniles comprising a population (calculated as J/total) and the proportion of breeding females that have released a brood (calculated as RB/(RB+B)) relative to the local degree of sea-ice breakout. In November 2006, mean values are given for the 'low' sea-ice category. All comparisons are restricted to data from the same month and year.

4.4.2. Abundance and distribution

4.4.2.1. Mesh size effects: 1mm vs 0.5mm

Estimates of abundance derived from a 1mm mesh size were strongly correlated to total (0.5mm mesh-derived) abundance ($R^2 = 0.96$, $p < 0.001$; Figure 4.9). Thus, we considered our use of 1mm fractions valid to explore overall patterns in abundance. Furthermore, population dynamic data revealed the minimum size of juveniles to be 2.5mm, therefore we considered the fraction of individuals excluded by a 1mm mesh more likely to reflect a longitudinal body orientation when passing through the sieve, rather than a particular (i.e. juvenile) component of the population.

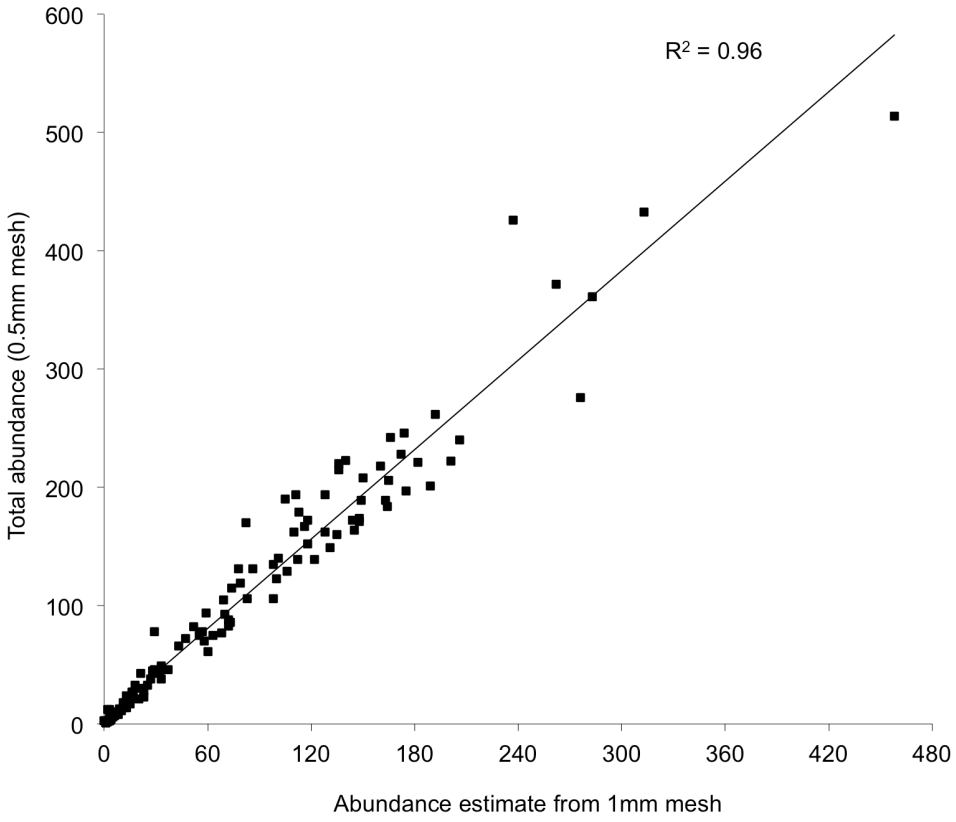

Figure 4.9: Correlation between *O. franklini* abundance estimates derived from a 1mm mesh size, and total abundance (i.e. derived from a 0.5mm mesh size).

4.4.2.2. Spatial and temporal patterns of abundance

O. franklini reaches astounding abundances in the shallow Antarctic benthos. The highest abundance of *O. franklini* found in a single core (sieved at 0.5mm) was 514 individuals, from Brown Bay Middle in December 2005. Extrapolating from the surface area sampled by a 10cm-diameter core, this correlates to a maximum density of approximately 65,400 individuals/m^2 of benthic sediment.

The abundance of *O. franklini* is highly heterogeneous, with significant variation revealed at all spatial scales examined, for all sampling periods tested. PERMANOVA revealed significant differences in abundance among plots at both Casey and Davis, indicating patchiness on scales as fine as 10-100m (Figures 4.10 and 4.11, Table 4.3). Variation in abundance was also significant among locations sampled within both of the regions (Figures 4.10 and 4.11, Table 4.3). However, the majority of variance in abundance at Casey was attributed to areas sampled (63-72%; Figure 4.10; Table 4.3a-c), whereas areas sampled at Davis did not differ significantly from each other. Rather, the majority of variation in abundance at Davis was attributed to location (82%; Figure 4.11; Table 4.3d).

The abundance of *O. franklini* also appears to fluctuate significantly in time. Differences in abundance among years were observed for all locations tested, with sampling year explaining between 22% and 84% of the variance in abundance (Figure 4.12; Table 4.4). However, the nature of these inter-annual fluctuations was clearly location-specific. For example, from the years 1997 to 2006 there appears to be a steady increase in abundance at both Brown Bay Middle and O'Brien Bay 2, yet a clear decrease in abundance at Wilkes (Figure 4.12).

Variation in abundance with sampling month was also expected, as population dynamic results revealed a strong annual influx of juveniles beginning in October and ending in January. Whilst the result of this test was not significant (Table 4.5), it was very close to the critical threshold of $p < 0.05$, and observation of the data indicated the expected pattern with lower average abundance in October compared to December (Figure 4.13). Given that this test was only feasible for a single location and year we cannot draw a robust conclusion either way; hence we deemed it prudent to conservatively remove any potential effect of sampling time from spatial analyses (see Section 4.3.2.2.).

Table 4.3: Results of 3-factor nested PERMANOVAs for spatial variation in *O. franklini* abundance at Casey (a-c) and Davis (d). Tests were restricted to data from the same 30-day sampling period to minimise confounding temporal effects.

a) Casey (Jan-Feb 2003)

Factor	df	MS	F-ratio	p-value	% total variance component
area	1	232.22	9.35	0.021	62.59
location (area)	6	24.72	16.51	<0.001	26.39
plot (location(area))	24	1.50	4.79	<0.001	5.38
residual	95	0.31			5.64

b) Casey (Nov-Dec 2005)

Factor	df	MS	F-ratio	p-value	% total variance component
area	1	124.20	9.93	0.032	69.79
location (area)	4	12.51	13.65	<0.001	19.31
plot (location(area))	18	0.92	3.63	<0.001	4.42
residual	69	0.25			6.48

c) Casey (Nov-Dec 2006)

Factor	df	MS	F-ratio	p-value	% total variance component
area	2	37.84	9.21	0.049	72.15
location (area)	3	4.15	9.68	<0.001	14.13
plot (location(area))	17	0.43	2.34	0.009	3.55
residual	65	0.19			10.17

d) Davis (Feb-Mar 2010)

Factor	df	MS	F-ratio	p-value	% total variance component
area	5	6.13	0.32	0.911	0
location (area)	18	19.31	20.91	<0.001	81.57
plot (location(area))	24	0.92	3.16	<0.001	6.62
residual	138	0.29			11.80

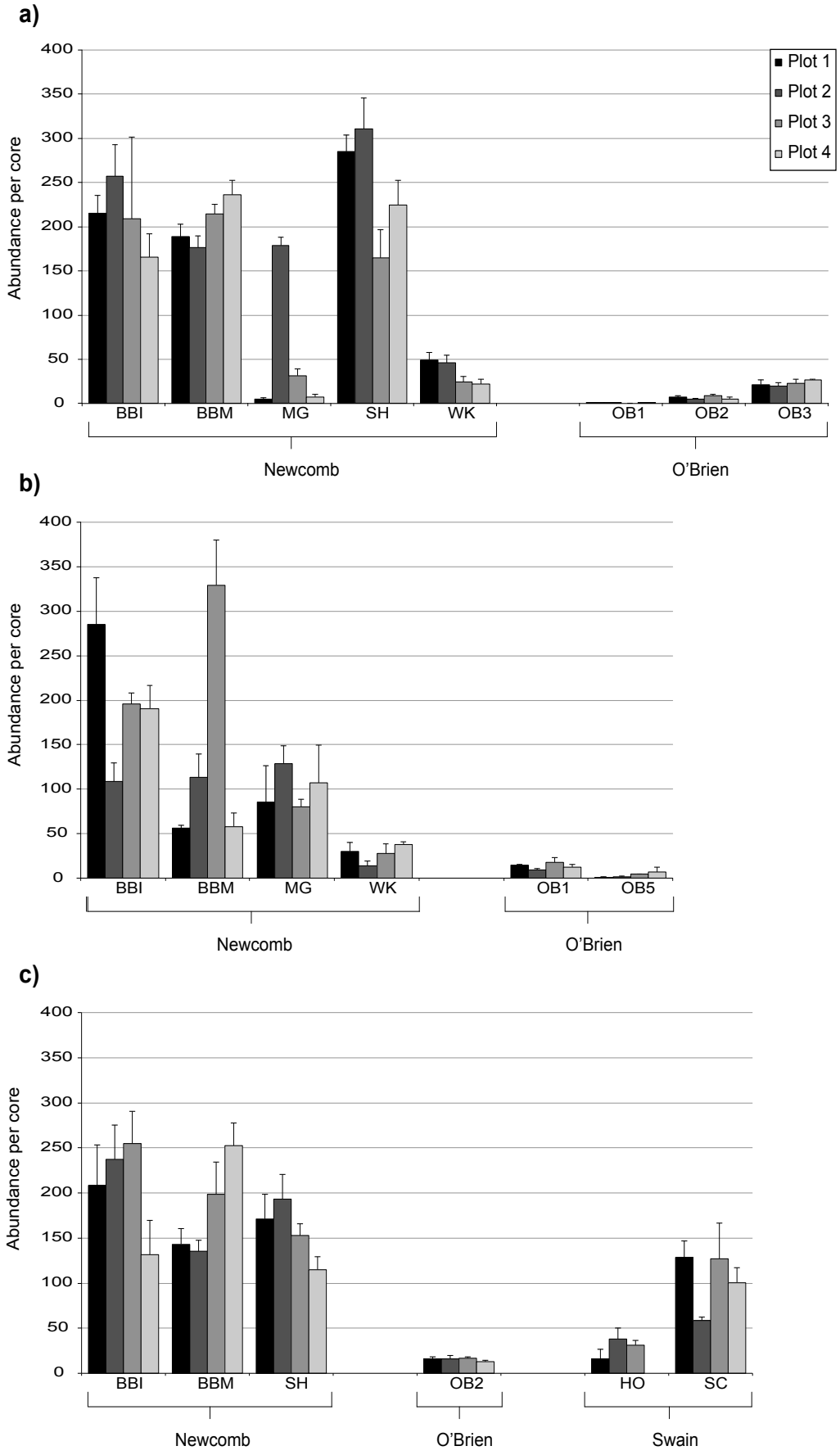

Figure 4.10: Mean abundance of *O. franklini* for each of four plots within locations at Casey during the sampling periods January 8th-February 8th 2003 (a), November 18th-December 16th 2005 (b), and November 8th-December 7th 2006 (c). Locations are subdivided into areas as indicated by square brackets labeled on the x-axis. Standard error bars are shown.

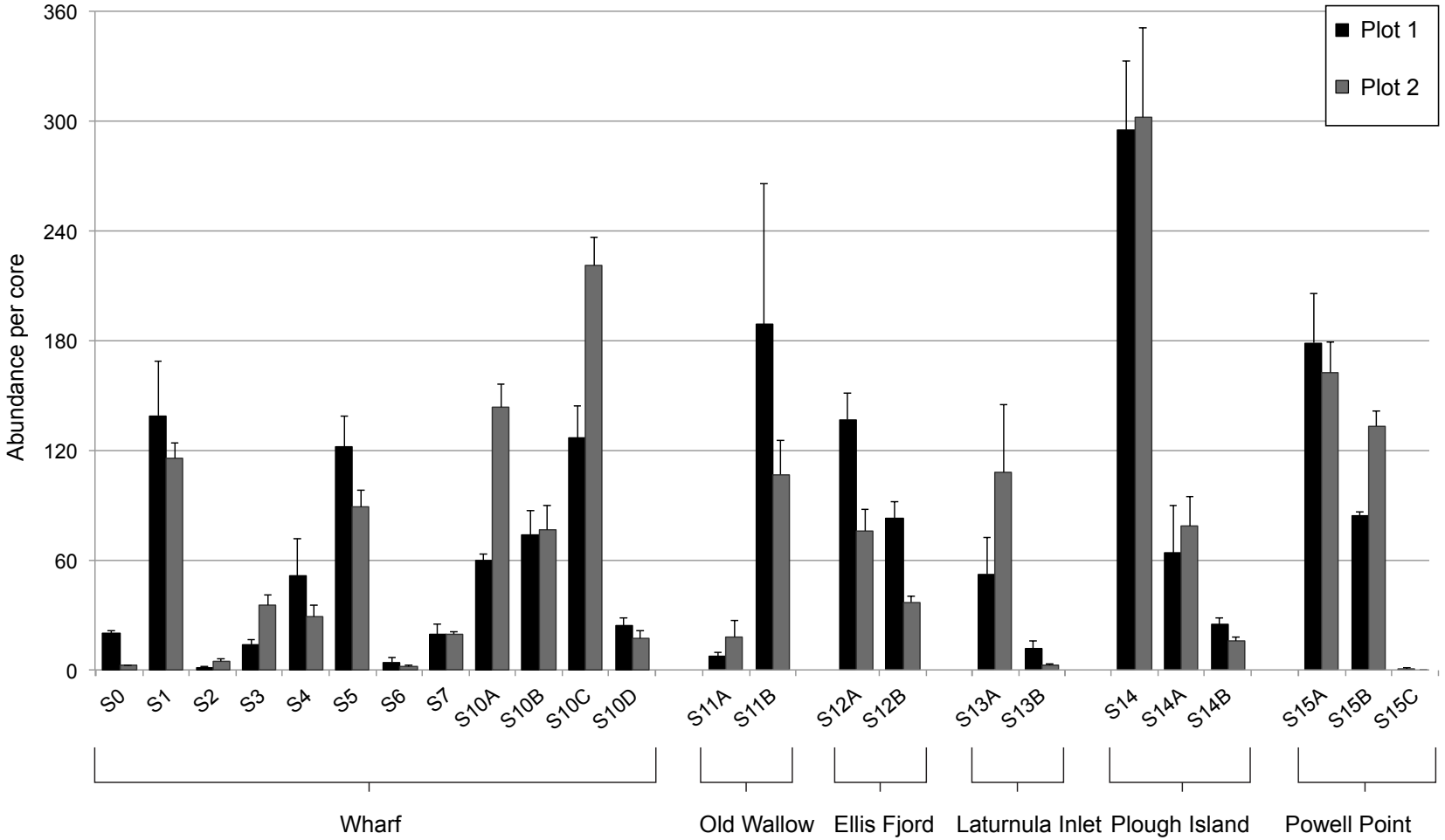

Figure 4.11: Mean abundance of *O. franklini* for each of two plots within locations at Davis (all sampled between February 12th and March 12th, 2010). Locations are subdivided into areas as indicated by square brackets labelled on the x-axis. Standard error bars are shown.

Table 4.4: Summary of results of single factor PERMANOVAs for inter-annual variation in *O. franklini* abundance.

Location	Month	Years compared	p-value from ANOVA	% total variance explained by year
BBM	December	1997, 1998, 2005	0.013	22.26
BBM	January	2003, 2004	0.065	22.19
OB2	November	1997, 1998, 2006	<0.001	67.70
WLK	December	1997, 2005	<0.001	66.60
WLK	January	1999, 2003, 2004	<0.001	76.41

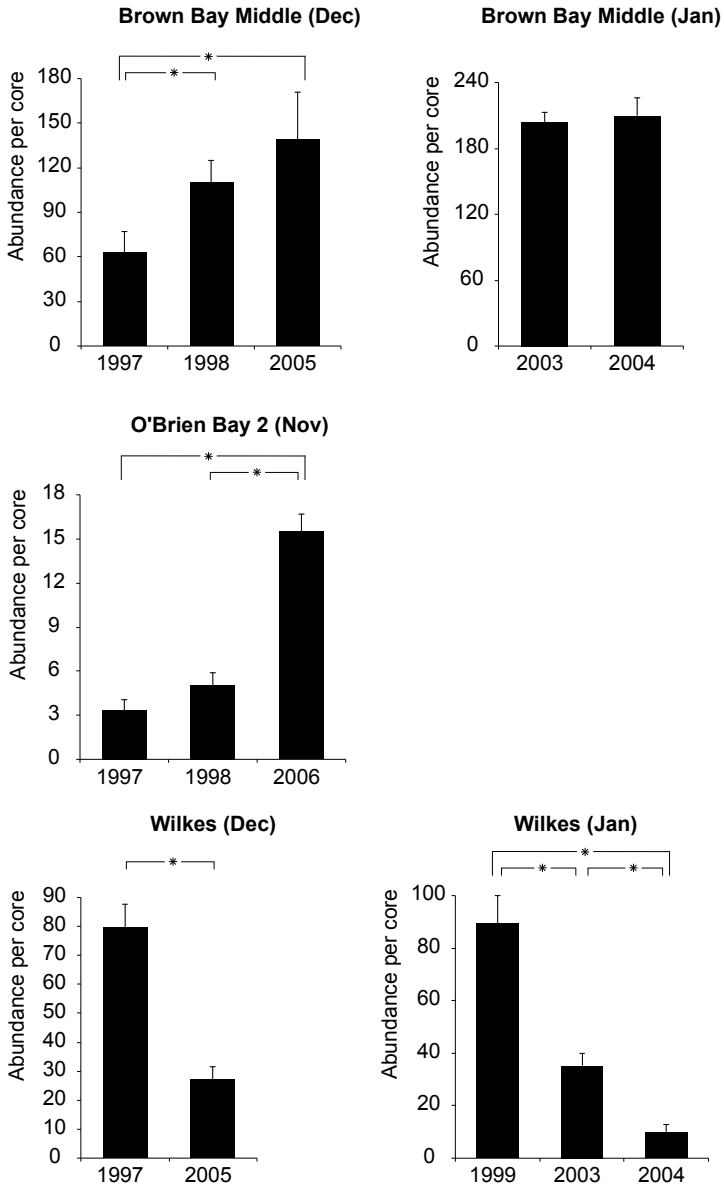

Figure 4.12: Mean abundance of *O. franklini* from cores (minimum n = 12) sampled in the same month across different years at Casey locations. Asterisks indicate significant differences (as determined by post-hoc pairwise comparisons). Standard error bars are shown.

Table 4.5: Summary of results of single factor PERMANOVA for monthly variation in *O. franklini* abundance.

Location	Year	Months compared	p-value from ANOVA	% total variance explained by month
BBM	1997	October, December	0.054	15.63

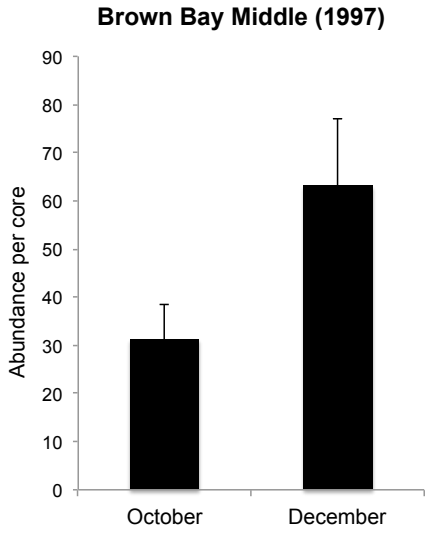

Figure 4.13: Mean abundance of *O. franklini* from cores sampled in October (n = 16) and December (n = 16) of 1997 at Brown Bay Middle. Standard error bars are shown.

4.4.2.3. Relationship between sediment properties and amphipod distribution

Spatial variability in sediment properties at Casey and Davis is correlated with spatial patterns of *O. franklini* abundance. ANOSIM revealed significant differences in sediments at the location scale at both Casey and Davis ($R > 0.6$, $p < 0.001$ for both regions). However, only at Casey were sediments found to vary significantly among areas (Casey: $R = 0.21$, $p = 0.004$, Figure S4.2; Davis: $R = 0.14$, $p = 0.06$, Figure S4.3), mirroring the result for spatial analysis of *O. franklini* abundance (see Section 4.4.2.2.). There were also differences in the overall variability of sediment properties present at Casey and Davis. Casey showed higher variability in trace metal bioavailability (Figure 4.14), whereas Davis was characterised by wider variability in grain size (Figure 4.15).

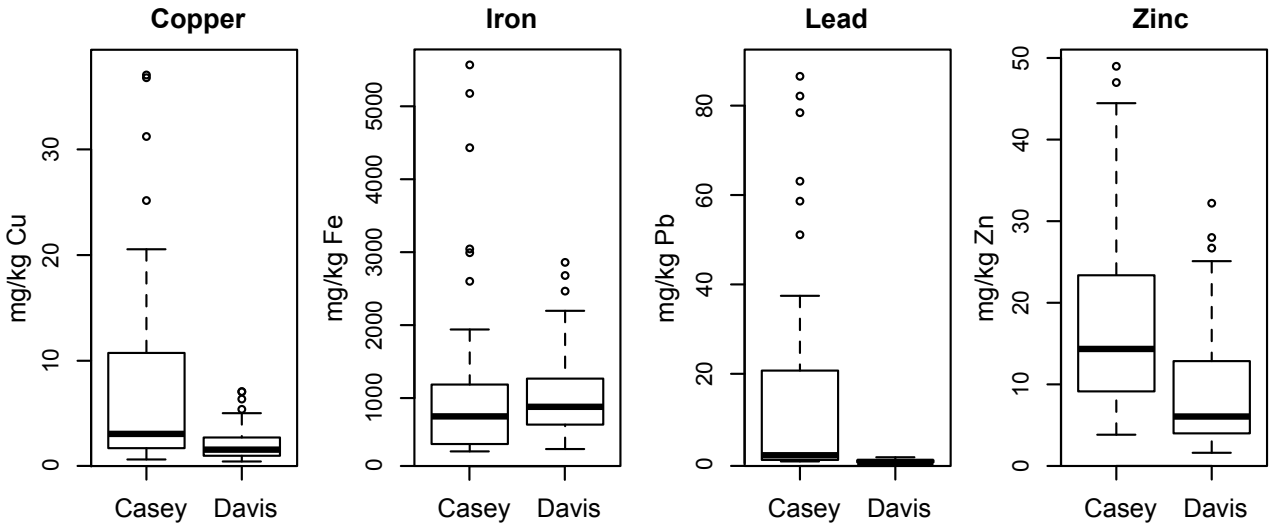

Figure 4.14: Boxplots of copper, iron, lead, and zinc concentrations in Casey and Davis sediments, showing the wider range of values measured at Casey.

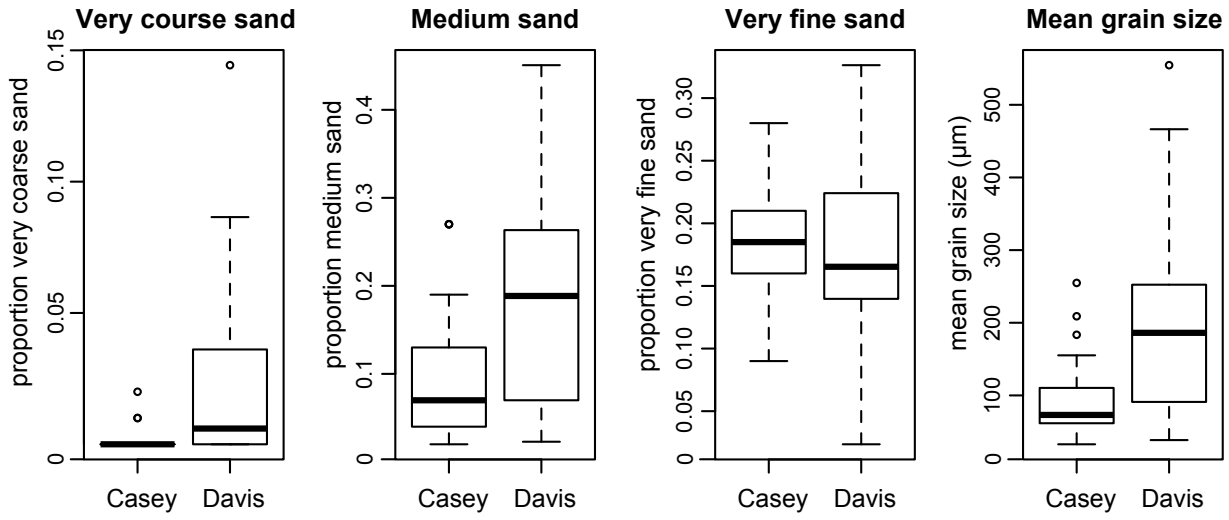

Figure 4.15: Boxplots of grain size for Casey and Davis sediments. Parameters are the proportion of grains that are very coarse, medium and very fine sands; and mean grain size.

For Casey and Davis data combined, a PCA ordination of all sediment parameters explained 60% of total environmental variation within the first two principal components. The resultant GAM explained 43% of total variation in abundance of *O. franklini*. This model predicted an increase in abundance with decreasing grain size, although optimum abundance occurred before grain size reached minimum values (Figure 4.16). Abundance was also predicted to increase with increasing TOC, with a peak in abundance occurring at moderately high values, before TOC reached its maximum (Figure 4.17). Higher abundances were also observed in sediments with generally higher trace metal concentrations, in particular Cu, Fe, Pb (Figure 4.18), Mg, Sb, Sn and Zn.

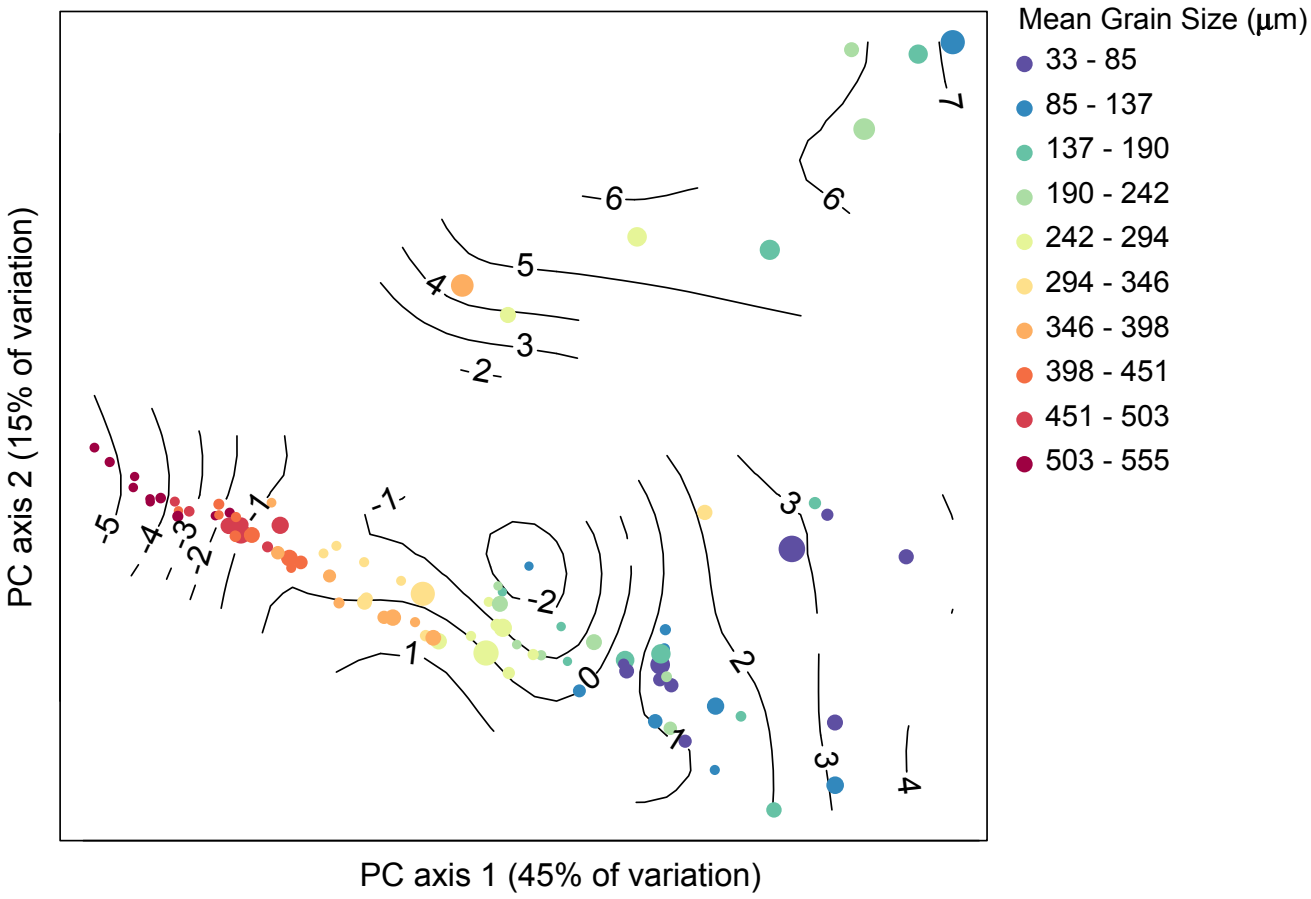

Figure 4.16: PCA ordination of sediment parameters for Casey and Davis data combined. The first two principal components account for 60% of environmental variation. Points are scaled by mean abundance of *O. franklini* and coloured by mean grain size (increasing from cool to warm colours). Contours represent predictions of square-root transformed abundance based on GAM (note that negative contours are the result of smoothing by GAM). Predicted abundance increases as mean grain size decreases, with maximum abundance at moderately small mean grain size. Lowest abundance is predicted for sediments with the largest mean grain size.

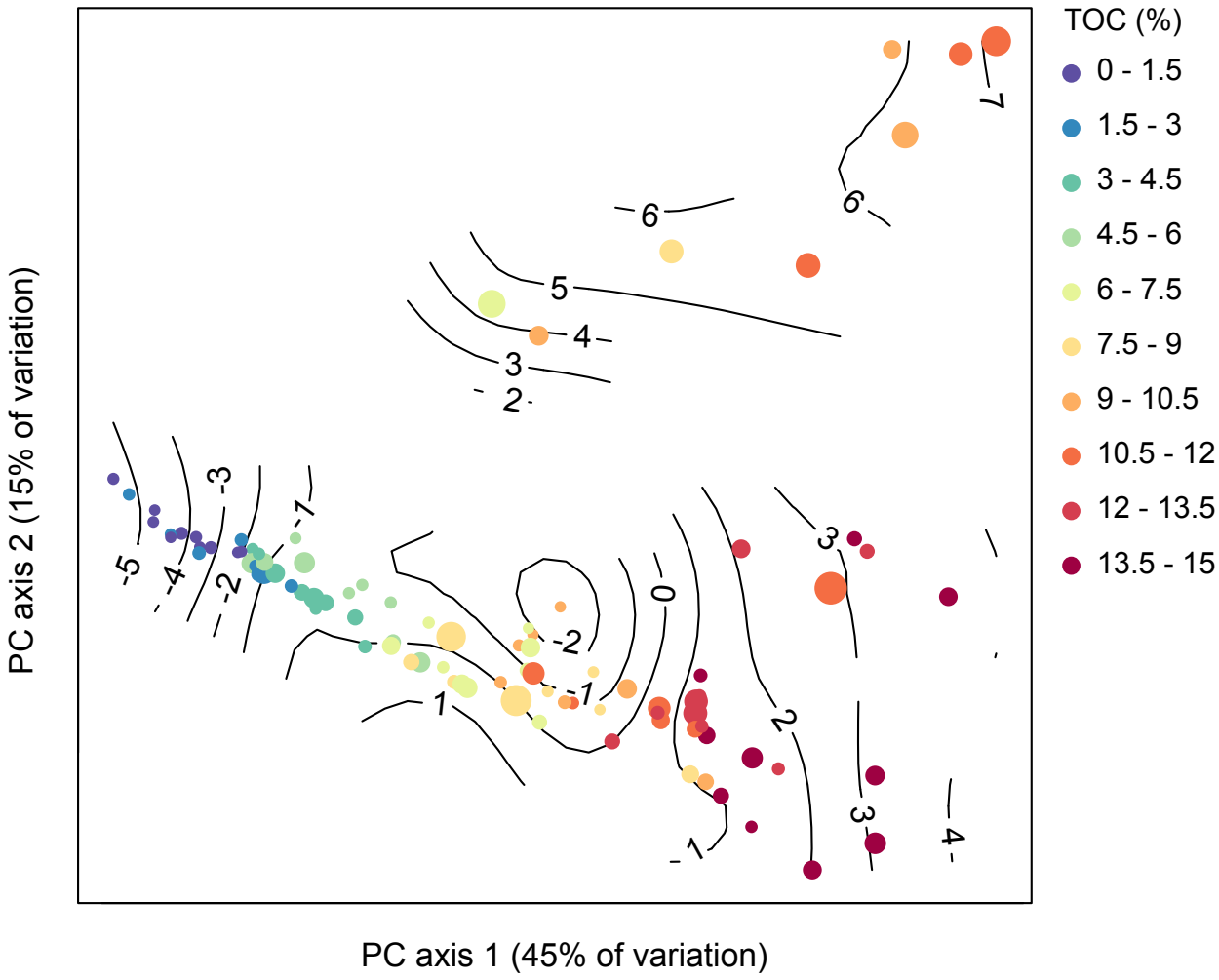

Figure 4.17: PCA ordination of sediment parameters for Casey and Davis data combined. The first two principal components account for 60% of environmental variation. Points are scaled by mean abundance of *O. franklini* and coloured by total organic carbon (TOC: increasing from cool to warm colours). Contours represent predictions of square-root transformed abundance based on GAM (note that negative contours are the result of smoothing by GAM). Abundance is predicted to increase with TOC content, peaking at moderately high (but not maximum) TOC. Lowest abundance is predicted for sediments with minimum TOC.

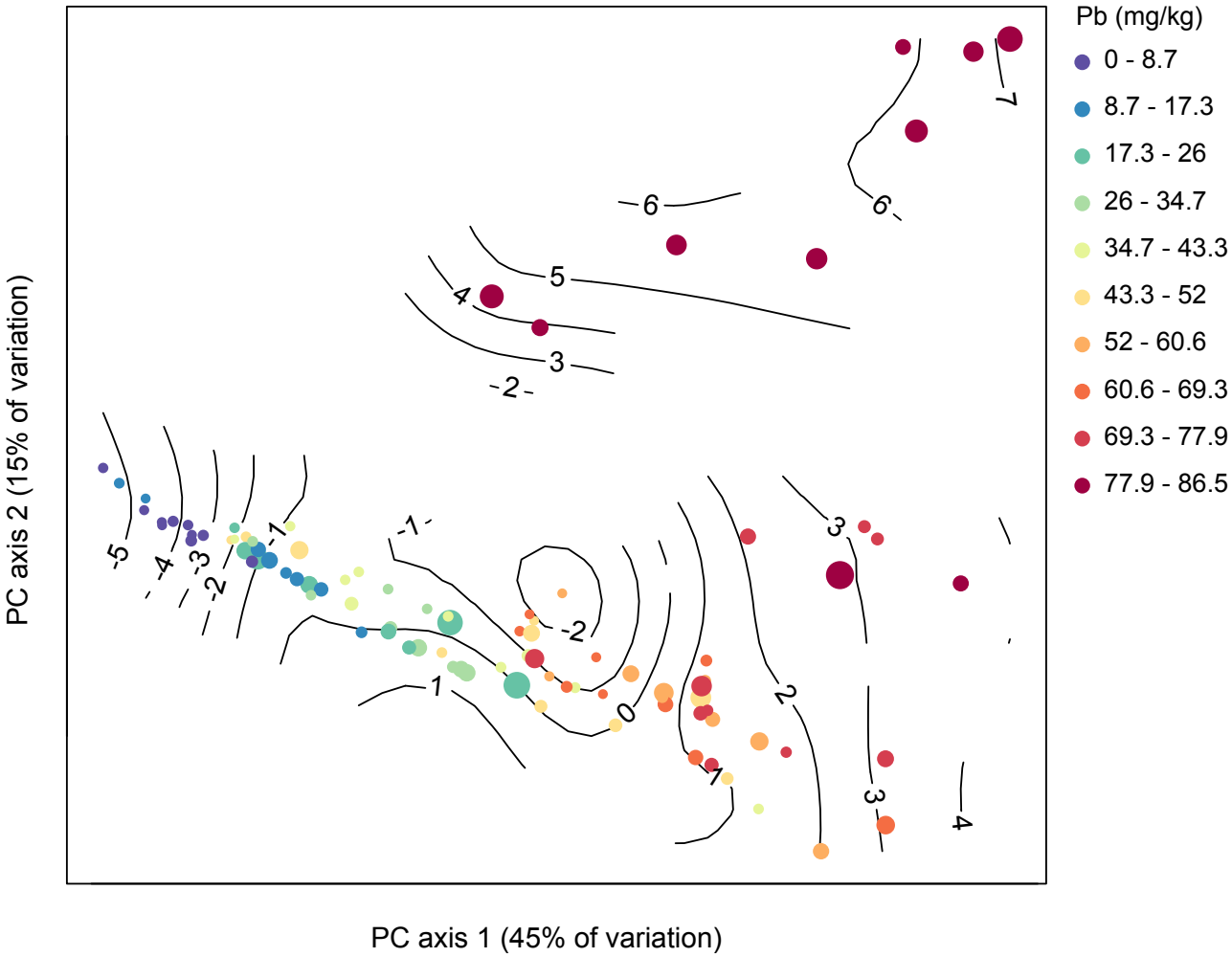

Figure 4.18: PCA ordination of sediment parameters for Casey and Davis data combined. The first two principal components account for 60% of environmental variation. Points are scaled by mean abundance of *O. franklini* and coloured by lead concentration (increasing from cool to warm colours). Contours represent predictions of square-root transformed abundance based on GAM (note that negative contours are the result of smoothing by GAM). An increase in abundance is predicted as the lead concentration of sediments increases. A similar pattern was observed for several other heavy metals (see Section 4.4.2.3.).

To determine if the observed relationship between sediment parameters and abundance was equally important at both of the study regions, PCAs were also generated for Casey and Davis data independently. For Casey, the first two components of a PCA explained 58% of the total variation in sediments and the resultant GAM explained 43% of the variation in abundance of *O. franklini*. This model predicted higher abundance in sediments with higher metallic bioavailability, supporting similar predictions generated from the combined dataset. The variables most responsible for this pattern at Casey were the heavy metals Cu, Fe, Pb (Figure S4.4), Sn and Zn. However, the relationship between *O. franklini* abundance and sediment grain size at Casey was less clear, with no strong correlation evident for any particular grain size parameter. For Davis, 67% of the total sediment variability was explained by the first two principal components and the corresponding GAM explained 49% of the variation in *O. franklini* abundance. This model suggested a complex relationship with the sediment, which in general supported all predictions from the combined model. Higher abundances were observed for sediments with higher TOC, lower grain size (Figure S4.5) and higher bioavailability of most trace elements.

4.5. DISCUSSION

4.5.1. Population dynamics of *Orchomenella franklini*

The population dynamics of *O. franklini* revealed several key traits that portray a life history considered well adapted to the polar environment, including seasonal breeding, longevity, extended brood incubation and low fecundity. Intraspecific differences in population structure were also observed, which highlight local spatial and temporal environmental fluctuations. Adaptation of *O. franklini* to nearshore Antarctic environmental conditions has important implications for the vulnerability of this species to climate change.

There is increasing evidence that the cold temperatures and predictable, seasonally fluctuating food supply of the polar regions should favour K-selected (Pianka 1970; or more specifically, A-selected: Greenslade 1983) life history strategies. These strategies are characterised by slower, delayed developmental processes,

longevity and reduced reproductive output. Compared to lower-latitude amphipod species (the vast majority of which die between the first and second year of life: Sainte-Marie 1991), *O. franklini* displays delayed reproduction (i.e. after two years of age) and considerable longevity (i.e. an approximately three year lifespan). The minimum four month brood incubation period observed for *O. franklini* also represents relatively slow development, characteristic of high latitude amphipods (see Rakusa-Suszczewski 1972; Klages 1993; Arndt & Swadling 2006). A mean of three and maximum of seven brooded eggs were found in *O. franklini*, which is in agreement with the prediction of low fecundity for A-selected strategies (see Greenslade 1983). However a brood size of 3-7 eggs is particularly low even for polar amphipods (see Sainte-Marie 1991) and the lowest to our knowledge recorded from the Antarctic. This may in part be explained by the relatively small body size (2.5-9mm) of *O. franklini* (see Sainte-Marie 1991) for comparison), as this study and many others have shown amphipod body size and brood size to be correlated (see Highsmith & Coyle 1991; Sainte-Marie 1991). The overall dominance of females observed across the *O. franklini* metapopulation represents yet another theorised adaptation of polar organisms. A population sex ratio in favour of females is a strategy believed to maximize breeding opportunity in adverse conditions (Sainte-Marie 1991; Arndt & Beuchel 2006).

Reproduction in *O. franklini* is seasonal, with egg incubation occurring over the winter. During late spring/early summer juveniles are released from the brood pouch, presumably timed to coincide with the Antarctic summer pulse of primary productivity. Speculations that scavenging and deposit-feeding brooders are largely uncoupled from Antarctic seasonality (e.g. Clarke 1988; Arntz *et al.* 1992; Arndt & Beuchel 2006) should therefore be regarded cautiously. *O. franklini* has long been considered a deposit-feeder (Knox 2007) and results from recent stable isotope work at Casey and Davis places *O. franklini* in a trophic position overlapping both deposit-feeders and scavengers (Gillies *et al.* In press). Strong seasonality in a deposit-feeder/scavenger may indicate a preference for seasonally variable stocks of fresh phytodetritus (Suhr *et al.* 2003; Mincks & Smith 2007). Indeed, carbon isotope values for *O. franklini* ($\delta^{13}C \approx$ -14.5%) indicate sea-ice algae as a likely food source (Gillies *et al.* In press). Other polar studies are also beginning to demonstrate seasonality in brooding taxa that feed within the sediment (e.g. detritivorous peracarids: Brandt 1996; various scavenging invertebrates: Obermüller *et al.* 2010). Clearly, benthic deposit-feeders and scavengers

may be more affected by seasonal productivity than previously thought, through the associated flux of phytodetritus to the seabed.

Adaptation to the seasonal availability of fresh phytoplankton may also help explain the spatial and inter-annual fluctuations in population structure revealed for *O. franklini* (albeit based on limited sample size). The timing of brood release in *O. franklini* appeared to differ between years, and while multi-year observations of reproduction for other Antarctic benthic marine invertebrates are rare (Grange *et al.* 2011), reports are emerging of strong inter-annual differences attributed to fluctuations in primary productivity (Stanwell-Smith & Clarke 1998; Chiantore *et al.* 2002; Grange *et al.* 2007). Indeed, the timing and intensity of primary productivity and flux of phytodetrital matter in Antarctic coastal waters is known to show strong inter-annual differences (Arrigo & van Dijken 2004; Smith *et al.* 2008). We also found preliminary evidence that the release of juveniles from the brood pouch may occur earlier in locations characterised by earlier sea-ice breakout, likely to result in an earlier phytoplankton bloom (see Ducklow *et al.* 2006). This raises the possibility that local environmental cues which signal the onset of summer productivity may contribute to the spatial and temporal heterogeneity of reproductive timing for *O. franklini*. Indeed, such cues are a common phenomenon in marine invertebrates (Lawrence & Soame 2004), although they remain largely unstudied in amphipods (Johnson *et al.* 2001).

Strong evidence of exponential growth for male and non-breeding female *O. franklini* was consistent with growth models generally accepted for amphipods (Welton & Clarke 1980; Highsmith & Coyle 1991). However, sigmoid models (e.g. von Bertalanffy and Gompertz) have typically been used to portray growth in polar amphipods (e.g. Brey & Clarke 1993; Poltermann 2000; Arndt & Beuchel 2006), most likely because the inclusion of breeding females in these studies yields a plateau in growth. Therefore, comparison of the growth rate in *O. franklini* with other polar amphipods was not possible. However, exponential growth at different temperatures in the temperate amphipod *Gammarus pulex* was investigated by Welton & Clarke (1980). The daily growth rate in *G. pulex*, as given by *b* in the equation

$$\log_e(\textit{length in mm}) = b(\textit{time in days}) + \textit{constant}$$

was found to be 0.0096 at 20°C, and 0.0027 at 5°C. In contrast, the daily growth rate for *O. franklini* (derived by dividing the monthly growth rate - see Section 4.4.1.1 - by the mean number of days in a month) was 0.00098, considerably lower than the cold-water

estimate for *G. pulex*, and an order of magnitude lower than the 20°C estimate. This provides preliminary evidence that growth in *O. franklini* is slow in accordance with predictions of slower growth at colder temperatures.

In summary, growth and reproduction in *O. franklini* reflect adaptations to the unique broad-scale conditions of the shallow Antarctic benthos, yet local fluctuations in the environment also appear to structure populations. Such environmental conditions will be affected by future climate change. Sea-ice dynamics in particular are already showing signs of change, with a reduction in the extent of coverage west of the Antarctic Peninsula, yet an increase in the extent and duration of coverage over other areas (see Thompson & Solomon 2002). Any changes in sea-ice dynamics are likely to disrupt the predictable summer phytoplankton bloom to which reproduction in *O. franklini* is so closely synchronised. Prolonged sea-ice breakout would likely increase the duration yet decrease the intensity of local phytoplankton (Schofield *et al.* 2010). The success of seasonal reproduction, which is designed to maximise new recruits over narrow periods of surplus food supply, could thus be compromised. Preliminary observations suggest that sea-ice breakout is involved in a cue for brood release in *O. franklini.* This is likely to reflect a strategy for coping with small-scale yearly fluctuations in sea-ice and therefore food supply. However, more profound changes in sea-ice in the future may therefore substantially influence brood release timing, with one potential outcome being premature brood release (i.e. before juvenile development is complete). Moreover, the specific cold-water adaptations observed in *O. franklini* such as slowed, delayed developmental processes may prove to be detrimental under a higher sea temperature regime, as is predicted for Antarctica (see Peck 2005; Clarke *et al.* 2007b). Ultimately, the future adaptability of these fine-tuned life history traits to a changing environment will determine the long-term success of *O. franklini*. However, the relatively long generation times revealed in this study suggest that *O. franklini* may have reduced evolutionary capacity to cope with any rapid change (see Berteaux *et al.* 2004).

4.5.2. Abundance and distribution of *O. franklini*

The maximum density estimate of 65,400 individuals/m^2 for *O. franklini* found in this study is to our knowledge the highest yet recorded for any Antarctic macrobenthic species. This data provides empirical evidence that *O. franklini* is indeed

one of the most numerically dominant members of the shallow Antarctic benthos. However, the abundance of *O. franklini* is highly heterogeneous on both temporal and spatial scales despite its widespread distribution, further highlighting the importance of local-scale structure in the Antarctic benthos. Overall there was evidence of a relationship between the distribution of *O. franklini* and sediment properties, partly explained by a deposit-feeding/scavenging lifestyle. The nature of this relationship differed with geographic region, again illustrating the close link between the ecology of *O. franklini* and local environmental conditions.

Significant variability in the abundance of *O. franklini* was observed over spatial scales as small as 10-100m (i.e. among plots) at both Casey and Davis, demonstrating a highly heterogeneous distribution in both regions. However, the majority of variation in abundance occurred on the largest spatial scale at Casey (i.e. among areas) yet on the intermediate scale at Davis (i.e. among locations). Abundance did not significantly differ among areas sampled at Davis. This may reflect discrete environmental differences: ANOSIM provided evidence that areas at Casey were characterised by different sediment attributes, while areas at Davis were completely heterogeneous with respect to sediments. However it may also partly reflect sampling design, as Davis was more intensively sampled than Casey, increasing the chance that differences among areas would be obscured by variability within areas.

Inter-annual fluctuations in the abundance of *O. franklini* were also apparent, although the nature of differences was clearly location-specific. This may reflect local fluctuations in food supply as a result of temporal sea-ice variability. While satellite images of sea-ice extent at Casey are available for the relevant years, they do not provide adequate resolution to discern location-specific differences (i.e. on the order of 100s of metres). However, the flux of phytodetritus to the benthos has been shown to differ markedly between years in other Antarctic regions (Smith *et al.* 2008). Furthermore, sea-ice and corresponding phytoplankton fluctuations in the Ross Sea have been directly linked to inter-annual variation in the abundance of several benthic species (Thrush & Cummings 2011). On a finer temporal scale, data enabling an investigation of the effect of month on *O. franklini* abundance was limited. Preliminary observations however suggested that an increase in abundance over the summer months is likely, particularly in light of the reproductive dynamics of *O. franklini.* Also, similar observations have been made in previous studies (see Tucker 1988).

The distribution of *O. franklini* appeared to be related to the grain size, TOC and trace element concentration of sediments. However, the relative influence of each factor must be interpreted with caution, as they are known to be correlated (smaller grain sizes generally have higher TOC and trace element bioavailability, see Goldberg *et al.* 1975; Horowitz 1985). Sediment grain sizes were much more variable at Davis than at Casey. Correspondingly, grain size parameters also exhibited a much stronger relationship with *O. franklini* abundance at Davis than at Casey. Abundance of *O. franklini* generally increased with decreasing sediment grain size, with maximum abundance predicted for moderately small (but not minimum) grain size. Abundance also increased with increasing TOC content, with maximum abundance predicted for moderately high (but not maximum) values of TOC. Although we cannot determine if one or both factors are driving this pattern given their correlative nature, both relationships are consistent with the deposit-feeding/scavenging lifestyle of *O. franklini*. Deposit-feeders are likely to favour the ingestion of smaller sediment particles (Taghon 1982), and both deposit-feeders and scavengers favour sediments with higher organic carbon content (for which there is some evidence from the Antarctic: Barry *et al.* 2003; Kim *et al.* 2007). The apparent avoidance of the finest grain sizes and highest TOC content is likely to reflect the fact that these sediment conditions can result in hypoxic or even anoxic sediments (Hyland *et al.* 2005). Hypoxic and anoxic sediments have previously been observed in the Antarctic nearshore environment (Stark *et al.* 2003b) and are known to be detrimental to many benthic species (Kvitek *et al.* 1998; Gray *et al.* 2002).

A higher abundance of *O. franklini* was consistently predicted for sediments with higher trace element concentrations, in particular metals. Rather than reflecting an affinity for high metal concentrations per se, it is likely a reflection of the affinity for fine-grained sediments, which correspondingly have higher trace element bioavailability. At Casey however, given the lack of any clear relationship between abundance and grain size, the stronger relationship observed for several heavy metals may indicate a genuine influence of metal bioavailability on distribution. Many crustaceans including amphipods are known to have the ability to regulate uptake and excretion of bioavailable trace metals in sediments (Rainbow 1997; Nassiri *et al.* 2000). It is possible that at Casey, where most metals reached higher concentrations than at Davis, an ability to regulate internal heavy metal levels affords *O. franklini* a competitive advantage in the more contaminated sediments.

Models based on PCA ordination of sediment parameters only explained up to half of the deviance in *O. franklini* abundance, indicating that additional environmental factors are most likely contributing to the observed distribution. Potential factors (which we could not explore due to insufficient data) include the extent of sea-ice cover (and therefore UV exposure), depth (although infaunal amphipods are less likely to exhibit depth zonation than other organisms: Arntz *et al.* 1994), hydrodynamics of the water column such as turbidity and current speeds, concentration of phytoplankton, and the density of other species that may have an effect via competition or predation. It is also possible that a large part of the unexplained distribution of *O. franklini* may simply reflect stochastic processes, such as ice disturbance (see Gutt 2000; Barnes & Conlan 2007).

Nonetheless, the discovery of discernable patterns in abundance with respect to sediment characteristics has important implications for our understanding of the Antarctic benthos. Until recently, there has been extremely limited empirical evidence of an effect of sediment type on Antarctic benthic assemblages, leading to assumptions that sediment was relatively unimportant in structuring the Antarctic benthos (Gutt 2000; Gutt 2007). However, a study of benthos in the Ross Sea found that faunal distributions were indeed governed strongly by seafloor features including sediment type (Barry *et al.* 2003). Our observations for *O. franklini* now support a relationship between sediment parameters and benthic species distribution for East Antarctica. However, it remains to be seen whether this relationship is stronger for deposit-feeder/scavengers, due to their substantial reliance on the sediment for survival. In any case, anthropogenic-induced changes predicted for the nearshore Antarctic environment are likely to affect the abundance and distribution of *O. franklini*. Ongoing pollution of the local environment (i.e. metal contamination) could potentially increase the competitive success of *O. franklini*, yet increased sediment anoxia through eutrophication (e.g. from sewage) could be detrimental. Through broader atmospheric effects, future climate change is predicted to affect the turbidity of oceanic waters (Harley *et al.* 2006; Department of Climate Change 2009), which is likely to alter sediment nutrients and grain sizes. This too could have an important influence on the future distribution and abundance of *O. franklini*.

4.5.3. Concluding remarks on the ecology of *O. franklini*

The sheer abundance of *O. franklini* revealed herein indicates its dominance (albeit patchy) of the shallow Antarctic benthos. Amphipods in general are regarded as a particularly successful group in the Antarctic and their success has been partly attributed to life history strategies "pre-adapted" to Antarctic conditions (Brandt 1999; De Broyer *et al.* 2003b). Our study supports this theory, as the life history of *O. franklini* was found to include many of the traits considered beneficial in a polar environment. However, traits that underpin the current success of *O. franklini* may also increase sensitivity to future environmental change. Given the ecological dominance of *O. franklini*, changes in the population dynamics, abundance or distribution of this species are likely to have a wider effect on the Antarctic nearshore benthic ecosystem.

Both aspects of this study have also illuminated how closely the ecology of *O. franklini* is tied to the local environment, in turn leading to intraspecific variability in population dynamics and distribution. The effect of environmental heterogeneity on individual species' population ecology has scarcely been addressed in the Antarctic benthos. Often observations from a single location and time are used to generalise ecological patterns, due to logistical restrictions on the spatial and temporal breadth of sampling such an extreme environment. While there is substantial literature exploring the impact of iceberg scouring on the benthic environment (e.g. Barnes 1999; Gutt 2001; Gerdes *et al.* 2003; Teixidó *et al.* 2007), this study provides an indication that sea-ice coverage and sediment characteristics may also be important factors affecting the environmental, and therefore biological heterogeneity of the Antarctic benthos. Observations for *O. franklini* suggest that reproductive timing, population structure, abundance and distribution may all vary with geographic location, as well as from year to year. Such spatial and temporal fluctuations should be an important consideration in future studies of Antarctic benthic organisms.

Chapter 5. General Discussion

5.1. SYNTHESIS

The aim of this thesis was to enhance our understanding of the genetic and ecological structure of Antarctic benthic invertebrate populations. This was achieved through a combination of DNA sequencing, microsatellite genotyping and ecological measurements, using common amphipod species as model organisms. Together the results of these studies illuminate several issues for Antarctic brooding invertebrates and the benthos in general, which are outlined below.

5.1.1. Heterogeneity in the Antarctic benthos

Until recently, the Antarctic benthos has been considered a relatively homogeneous ecosystem. Studies have emphasised the stable, uniform environmental conditions prevailing around the Antarctic coast, the importance of the strong circumpolar currents for dispersal and the prevalence of species with apparently circum-Antarctic distributions (e.g. Hedgpeth 1970; Dell 1972; White 1984; Arntz *et al.* 1994). Whilst the heterogeneity of Antarctic benthic habitats and faunal communities have more recently been acknowledged (e.g. Raguá-Gil *et al.* 2004; Gutt 2007), a considerable degree of intraspecific heterogeneity remains overlooked, as revealed by the results of this thesis for Antarctic benthic amphipods.

Over the broadest spatial scale investigated, DNA sequencing indicated that two apparently circum-Antarctic sibling amphipod species are a complex of seven morphologically cryptic species (Chapter 2). This adds to a growing body of literature reporting divergent genetic lineages indicative of cryptic species in Antarctic benthic fauna (see Janosik & Halanych 2010). Furthermore, each of the cryptic species reported in Chapter 2 was found to have a unique geographic distribution (only one of them truly 'circum-Antarctic') and contrasting levels of intraspecific genetic diversity, possibly reflecting different isolation and recolonisation processes associated with glaciation

cycles. Such cryptic species diversity possibly represents the most important level of Antarctic benthic heterogeneity uncovered by the research of this thesis, as it highlights our current misunderstanding of biodiversity and species distributions in Antarctica. Inaccurate biodiversity estimates compromise our ability to monitor both the effectiveness of management strategies and the impacts of environmental change (Hogg *et al.* 1998; Plaisance *et al.* 2011).

Strong intraspecific genetic heterogeneity was also apparent across broad regions of the Antarctic coast both for nearshore and deeper shelf-dwelling amphipod species. Populations within cryptic *Eusirus* species were found to be genetically isolated over 100s to 1000s of kilometres (Chapter 2), and high genetic subdivision between Casey and Davis populations separated by ~1000 kilometres was revealed for *Orchomenella franklini* (Chapter 3). In both cases, limited long-distance gene flow is most likely a reflection of the restricted dispersal conferred by a brooding mode of development in amphipods, which precludes any pelagic larval phase. While population subdivision is therefore not surprising for brooding species, other Antarctic brooders have been observed to maintain considerable gene flow over 100s to 1000s of kilometres, possibly aided by strong circumpolar currents (Mahon *et al.* 2008; Leese *et al.* 2010). Genetic isolation of *O. franklini* populations over a broad scale also appears to be enhanced by directional selection, indicating localised adaptation within populations at Casey and Davis (Chapter 3). Together with the different levels of genetic diversity observed for the two regions, this suggests that varied environmental conditions around the Antarctic coast play an important role in structuring Antarctic benthic populations. Further support for the importance of regional environmental variation in structuring Antarctic benthic populations was demonstrated by the different sediment attributes characterising Casey and Davis, which appeared to influence the distribution of *O. franklini* to different degrees (Chapter 4).

This research also identified heterogeneity in amphipod populations on local scales (i.e. within regions). Genetic population differentiation was evident over 10s of kilometres in *O. franklini* and dispersal in this species appears to be restricted to adjacent populations (Chapter 3). This was one of the first reports of restricted gene flow over such a small distance in the Antarctic benthos. Ecological population structure was also found to vary over local scales, with differences in the reproductive timing of *O. franklini* potentially reflecting variation in local sea-ice conditions

(Chapter 4). Moreover, the abundance of *O. franklini* was highly heterogeneous on scales as fine as 100s of metres (Chapter 4). This was found to correlate to local variability in sediment grain size, total organic carbon (TOC) content and trace metal bioavailability, providing evidence that Antarctic benthic populations are potentially influenced by sediment features - a concept that has been under some doubt (see Gutt 2007). Together these genetic and ecological results highlight the importance of local scale variability in structuring benthic communities, yet in Antarctica, this scale is perhaps the least understood.

Finally, ecological heterogeneity was evident on a temporal scale. There was preliminary evidence in *O. franklini* that the timing of brood release and the juvenile composition of populations differed between years at several locations (Chapter 4). Moreover, significant inter-annual fluctuations in the abundance of *O. franklini* were observed, yet the nature of these fluctuations differed with location. It is suggested that these temporal differences in population ecology are driven by local sea-ice dynamics and primary production, which show profound inter-annual fluctuations in nearshore Antarctic waters (e.g. Arrigo & van Dijken 2004; Ducklow *et al.* 2006).

In summary, the research in this thesis has highlighted the heterogeneous nature of the Antarctic benthos, as revealed by both nearshore and shelf-dwelling amphipod species. Amphipod populations are structured over a variety of spatial and temporal scales, ranging from cryptic genetic lineages on a circum-Antarctic scale, to temporal fluctuations in population dynamics. Although Antarctic benthic studies have begun to address the overall heterogeneity of species assemblages, attention must also be focused at the intraspecific level to ensure a comprehensive understanding of the dynamic structure of the Antarctic benthos.

5.1.2. Speciation in the Antarctic benthos

The studies within this thesis shed light on the high speciation of amphipods and more generally peracarid crustaceans in Antarctica. The discovery of cryptic speciation in *Eusirus* species (Chapter 2) supports the theory that historical glaciation processes have acted as a "diversity pump" in the Antarctic by driving benthic populations into physically-isolated, ice-free refugia, thereby promoting rapid allopatric speciation (see Clarke 1996). It is believed that brooding organisms such as peracarid crustaceans may have been particularly susceptible to such glacial speciation processes. Their lack of

pelagic larvae would inhibit the ability to rapidly and widely recolonise available habitats post-glaciation, thus prolonging population isolation (Thatje *et al.* 2005; Wilson *et al.* 2009). Certainly, many of the reports of cryptic species diversity in the Antarctic benthos are for taxa that brood offspring (e.g. Held & Wägele 2005; Raupach & Wägele 2006; Mahon *et al.* 2008; Lörz *et al.* 2009; Wilson *et al.* 2009).

In addition to historical processes, modern-day gene flow is also likely to play a role in driving the speciation of amphipods and other brooding taxa in Antarctica. Severely limited gene flow between populations as a result of restricted dispersal was demonstrated for cryptic *Eusirus* species and *O. franklini* in Chapters 2 and 3. In both studies the differentiation of populations over large distances (i.e. > 100km) indicated such a degree of genetic isolation that allopatric speciation was considered likely to occur in the future. Indeed, restricted gene flow due to brooded development has been invoked by other authors to explain why groups such as the peracarid crustaceans are particularly speciose in Antarctica (Brandt 1999; De Broyer *et al.* 2003b; Held & Leese 2007). Furthermore, the evidence of selection contributing to genetic differentiation in *O. franklini* (Chapter 3) suggests that adaptation to local environments may also be a key mechanism driving high speciation rates in the Antarctic benthos.

It has been argued that the species radiations observed for many Antarctic peracarid groups have resulted from successful life history strategies inherently well adapted to polar conditions (Brandt 1999; De Broyer *et al.* 2003b). Observations for *O. franklini* did indeed reveal a suite of traits considered beneficial in the Antarctic environment, including delayed reproduction, longevity, low fecundity, and an annual brood release coinciding with summer phytoplankton blooms (Chapter 4). Moreover, the sheer numerical dominance of *O. franklini* in nearshore sediments implies that these ecological strategies allow it to thrive in the Antarctic environment. It therefore seems a valid notion that peracarid crustaceans such as amphipods boast a certain ecological success in the Antarctic, which has afforded them the opportunity for adaptive radiation contributing to the high species diversity and endemicity observed today.

5.1.3. Sensitivity of Antarctic benthic species to environmental change

The vulnerability of Antarctic species to future climate change is well recognised (Peck 2005) and results from this thesis highlight the potential risks. The low genetic connectivity observed between amphipod populations (Chapters 2 and 3)

may hamper the ability of these species to evolve effectively on a broad scale (see Hogg *et al.* 1998). Such restricted gene flow is likely to facilitate the localised adaptation of populations, yet limit the dispersal of advantageous alleles between populations for species adaptation to widespread change (Levins 1964; Slatkin 1987). Relatively long generation times observed for *O. franklini* (Chapter 4) as well as for other Antarctic benthic invertebrates (e.g. Wägele 1987b; Klages 1993) also raises doubt over the ability of these organisms to adapt rapidly (see Berteaux *et al.* 2004). This is of concern because areas of Antarctica are currently undergoing some of the most rapid climatic changes on earth (Vaughan *et al.* 2003).

Perhaps a more promising result with regard to the evolutionary potential of Antarctic benthic amphipods was the discovery of a considerable degree of overall genetic diversity in both *Eusirus* species and *O. franklini* (Chapters 2 and 3), challenging perceptions that high latitude fauna have depressed genetic variation (Hewitt 2000; Maggs *et al.* 2008). This is important because genetic diversity ultimately provides the raw material for any future adaptation (Lande & Shannon 1996; Bowen 1999). However, microsatellite variation in *O. franklini* did provide evidence of a loss of genetic diversity associated with anthropogenic pollution (Chapter 3). While this was considered a preliminary result, it certainly suggests a potential role for genetic monitoring of ongoing local anthropogenic impacts in the Antarctic.

Ecological strategies finely tuned to the unique conditions that presently characterise Antarctic marine environments may also increase species' vulnerability to future changes. Reproduction in *O. franklini* was observed to be synchronised with the seasonality of primary production in Antarctic waters (Chapter 4), with timing potentially driven by environmental cues related to sea-ice coverage. Any disruption to this phenological relationship due to climate change (see Moline *et al.* 2008) may have dire consequences for recruitment success in *O. franklini*, as well as other Antarctic species with seasonal reproduction (e.g. Bregazzi 1972; Sagar 1980; Stanwell-Smith & Clarke 1998). Furthermore, increased storm activity predicted for coastal waters as a result of climate change (Harley *et al.* 2006) is likely to alter sediment grain sizes and may thereby affect the distribution and abundance of *O. franklini*, due to its apparent affinity for finer grains (Chapter 4). *O. franklini* currently dominates many nearshore Antarctic benthic communities, meaning a change in its distribution or abundance is likely to affect other species occupying this habitat.

5.1.4. Implications for conservation

One of the broader goals of this thesis was to help inform planning for future management of the Antarctic benthic ecosystem. Results from the studies herein provide insight relevant to the design of Marine Protected Areas (MPAs) planned for Antarctic waters (see CCAMLR 2005). One of the key design priorities for MPAs is to adequately represent the biodiversity distributed within a region (ANZECC Taskforce on Marine Protected Areas 1998). Results from Chapter 2 demonstrate that the current estimates of species numbers and distributions in the Antarctic are erroneous, and this should be considered when assessing the inclusiveness of proposed MPAs. For example, it should not be assumed that a given MPA will contain at least some part of the distribution of the many 'circumpolar' benthic taxa, as these taxa could in fact be a complex of cryptic species, each with a unique geographic distribution (Raupach *et al.* 2007; Rogers 2007; Brandão *et al.* 2010).

Another key design principle recommended for MPAs is to ensure the ecological connectivity of species (ANZECC Taskforce on Marine Protected Areas 1998). Results from this thesis suggest that for some Antarctic brooders, connectivity is unlikely to be maintained between populations in protected areas spaced more than 100km apart. Furthermore, evidence of stepping-stone dispersal (Chapter 3) implies that the extinction of any one population is only likely to be replenished by adjacent populations, i.e. in the order of kilometres. Obviously it is unrealistic to expect all future MPAs in Antarctica to have such narrow spacing. However, the recent recommendation that MPA networks incorporate a range of different spacings (University of Queensland Ecology Centre 2009) certainly seems like a worthy option that should be considered for areas of the Antarctic.

Although it has not been at the forefront of marine conservation planning, protection of genetic diversity should be an important consideration for any management strategy, in order to ensure long-term species persistence (Hogg *et al.* 1998; Bell & Okamura 2005). Genetic diversity in *O. franklini* was found to be higher for Davis populations than those from Casey (Chapter 3), which possibly reflected higher environmental heterogeneity at Davis. Similarly, reports of populations with contrasting levels of genetic diversity are emerging for other Antarctic benthic invertebrates, often attributed to unique Antarctic environmental processes (e.g. Arango *et al.* 2010; Demarchi *et al.* 2010; Leese *et al.* 2010). It may therefore be particularly

important in the Antarctic to consider the genetic diversity of populations contained within proposed protected areas, since inadvertently conserving those with lower genetic diversity could compromise species' resilience to local catastrophe and environmental change (Bell & Okamura 2005).

5.2. FUTURE DIRECTIONS

The research in this thesis has significantly enhanced our understanding of the structure and ecology of Antarctic benthic species, yet has also identified several crucial directions for future research that will provide further insight and help ensure adequate management planning.

5.2.1. DNA taxonomy

The substantial cryptic speciation revealed herein highlights our current misunderstanding of the true biodiversity of the Antarctic benthos. That the two morphological amphipod species found to harbour cryptic species were considered well studied and anatomically giant is of particular concern. Clearly, the true species diversity of the Antarctic benthos is likely to remain hidden without increased and ongoing use of molecular tools to help study taxonomic relationships. Accurate taxonomic knowledge not only underpins biodiversity estimates and therefore our ability to monitor changes due to climate change (Hogg *et al.* 1998), it is also crucial to help reveal true species distributions, identify invasive species, explore trophic relationships and other ecological interactions, and inform management decisions (see Féral 2002; Sites & Marshall 2003; DeSalle & Amato 2004). Cryptic speciation is a common phenomenon in marine environments generally (Knowlton 1993), and biodiversity underestimates are considered a problem for even the most rigorously studied habitats, such as coral reefs (Plaisance *et al.* 2011). That the Antarctic is less sampled and appears to have a wealth of cryptic species derived in part from unique glacial processes means that embracing DNA-based taxonomic techniques may be especially important for understanding Antarctic diversity.

The keystone of DNA taxonomy is the concept of developing a universal DNA "barcode" for routine species identification, a concept that has generated both

excitement and controversy. Certainly, there are strong arguments against the complete replacement of traditional morphological taxonomy with DNA barcoding (Ebach & Holdrege 2005; Will *et al.* 2005). However, if used as a complementary tool that can help delimit species when diagnostic morphological traits are too subtle to discern reliably, the merits of DNA barcoding are clear (DeSalle *et al.* 2005; Bucklin *et al.* 2010). The cytochrome c oxidase subunit 1 (COI) gene region has shown considerable promise as a candidate DNA barcode (Hebert *et al.* 2003a), although it is not appropriate for all taxa (see Meyer & Paulay 2005). Internal transcribed spacer (ITS) regions have also been advocated for their use in species delimitation (Chu *et al.* 2001; Yao *et al.* 2010) and results from this thesis certainly illustrate some of the advantages of ITS2 over COI for amphipods (Chapter 2). Ultimately however, the best option (where time and finances permit) will be the use of DNA sequences from several molecular regions to help illuminate species boundaries (Edwards & Beerli 2000; Neigel *et al.* 2007).

Any molecular attempts to improve estimates of species diversity in the Antarctic are likely to be futile if not accompanied by increased sampling efforts. Rarefaction techniques employed in the research of this thesis indicated that significant DNA sequence diversity remained to be uncovered with further sampling, and similar results have been found in other Antarctic studies (e.g. Wilson *et al.* 2009). Indeed, growing discoveries of previously unrecognised species in the Antarctic have been attributed largely to enhanced sampling efforts in recent times (Brandt *et al.* 2007a; Bucklin *et al.* 2010), as well as increased molecular research (Griffiths 2010). Fortunately, recent collaborative initiatives such as the Census of Antarctic Marine Life (CAML) and Barcode of Life (BOLD) projects should increase both the sampling extent and DNA barcoding of Antarctic fauna (Grant & Linse 2009), to help address our current misunderstanding of Antarctic species diversity and distributions.

5.2.2. Temporal variability

Due to the strong seasonality of sea-ice, sunlight and phytoplankton in the Southern Ocean, the vast majority of studies investigating temporal dynamics in Antarctic marine faunal ecology have focused on intra-annual variability. While this has led to important discoveries of seasonal breeding in Antarctic organisms (with results from Chapter 4 a prime example), there has been little investigation of inter-annual

dynamics. Recent observations of the flux of phytodetrital matter to benthic sediments in the West Antarctic Peninsula have indicated greater inter-annual than seasonal variability (Smith *et al.* 2008), suggesting that the exploration of inter-annual ecological patterns may be particularly important for benthic fauna. Both the abundance and population structure of *O. franklini* showed evidence of inter-annual variability (Chapter 4), although low sample size prevented statistical testing. However, inter-annual differences in population abundance and reproductive condition are also coming to light in other Antarctic benthic species (e.g. scallop: Chiantore *et al.* 2002; seastar: Grange *et al.* 2007; polychaetes and crustaceans: Thrush & Cummings 2011). Invariably this has been attributed to local changes in sea-ice and phytoplankton dynamics, suggesting that exploring these patterns further will be crucial to advance our understanding of potential responses to future climate change.

The discovery of inter-annual differences in the ecological dynamics of *O. franklini* begs the question: how might this affect the genetic diversity and connectivity of populations? Unfortunately, exploring temporal variation in genetic structure was beyond the scope of this thesis, yet it certainly warrants future research in the Antarctic. Temporal genetic structure has not received as much attention as spatial genetic structure in marine studies generally (Heath *et al.* 2002; Bergek & Olsson 2009), despite having the potential to provide substantial insight into microevolutionary processes (Waples 1989). When explored, temporal fluctuations in genetic structure have shed light on effective population size (Heath *et al.* 2002), variability in reproductive success (Lundy *et al.* 2000; Olsen 2002) and localised adaptation (Hilbish 1985). In the Antarctic benthic ecosystem, monitoring changes in the genetic diversity and differentiation of populations over time could be incorporated with ecological data to help elucidate the effect of sea-ice and phytoplankton fluctuations on local population structure.

5.2.3. Population connectivity and spatial management

This thesis presents one of the first thorough investigations of genetic population connectivity over a range of scales in the Antarctic benthos (Chapter 3). The use of an ecologically dominant and widespread species (*O. franklini*) as the model organism renders the results of this study particularly relevant for future MPA planning. However, for MPAs in the Southern Ocean to achieve the "comprehensiveness,

adequacy and representativeness" required of them (ANZECC Taskforce on Marine Protected Areas 1998; CCAMLR 2005), information on population connectivity needs to be gathered from a wide range of other Antarctic benthic taxa. Although a number of studies have explored genetic population structure in other Antarctic benthic organisms, most have focused on connectivity across abyssal depths or the Polar Front (e.g. Linse *et al.* 2007; Hunter & Halanych 2008; Wilson *et al.* 2009). These are only likely to be informative for the connectivity of MPAs situated across similar hydrographic and environmental features. Also, the frequent identification of cryptic species in Antarctic benthic studies means that much of the existing genetic data actually represents interspecific structure.

The need to address this paucity of intraspecific genetic knowledge has already been recognised by authors advocating increased use of highly variable markers on Antarctic benthic fauna (Gaffney 2004; Held & Leese 2007). Less emphasis has been placed on the need to explore genetic structure over a range of geographic scales in the Antarctic. However, the importance of this was illustrated in Chapter 3 by the variety of processes discovered to structure *O. franklini* populations over different scales (i.e. panmixia over 100m, stepping-stone dispersal over 1-30km, genetic isolation and local adaptation over 1000km). In fragmented marine environments (as which the Antarctic benthos is gaining recognition), exploring gene flow over multiple scales will be especially important to help determine appropriate designs for spatial management (Palumbi 2003).

While the genetic structure revealed for *O. franklini* is likely to be relevant for other brooding benthic invertebrates, generating data for species with diverse life history strategies is crucial to avoid a biased view of population structure (Levin 2006). For the same reason, investigating taxa that represent different trophic niches and inhabit different environments will also be important. Finally, our understanding of connectivity in the Antarctic benthos will progress further when genetic research can be interpreted in light of behavioural and ecological studies. Ultimately, this will provide the most robust scientific foundation for future management planning.

5.3. CONCLUSION

Recently, a review of the current state of knowledge on life in the Southern Ocean identified several key priorities for research: “improved estimates of Antarctic marine biodiversity and better understanding of ecology…. as well as the potential faunal response to climate change” (Griffiths 2010). This thesis has provided insight on all of these issues, using one of the most dominant faunal groups of the Antarctic benthic ecosystem. It is essential that future studies and management plans consider the large degree of heterogeneity revealed herein for the Antarctic benthos over a range of spatial and temporal scales. Anthropogenic activities are having an increased impact on the once pristine Antarctic marine environment; we therefore have a narrow window of opportunity to better understand and preserve the vulnerable Antarctic benthos.

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Appendices

Appendix I contains all supplementary figures and tables for Chapter 2.

Appendix II contains all supplementary figures and tables for Chapter 3.

Appendix III contains all supplementary figures and tables for Chapter 4.

Appendix IV contains the published version of Chapter 2, as it appears in *Molecular Ecology*.

APPENDIX I

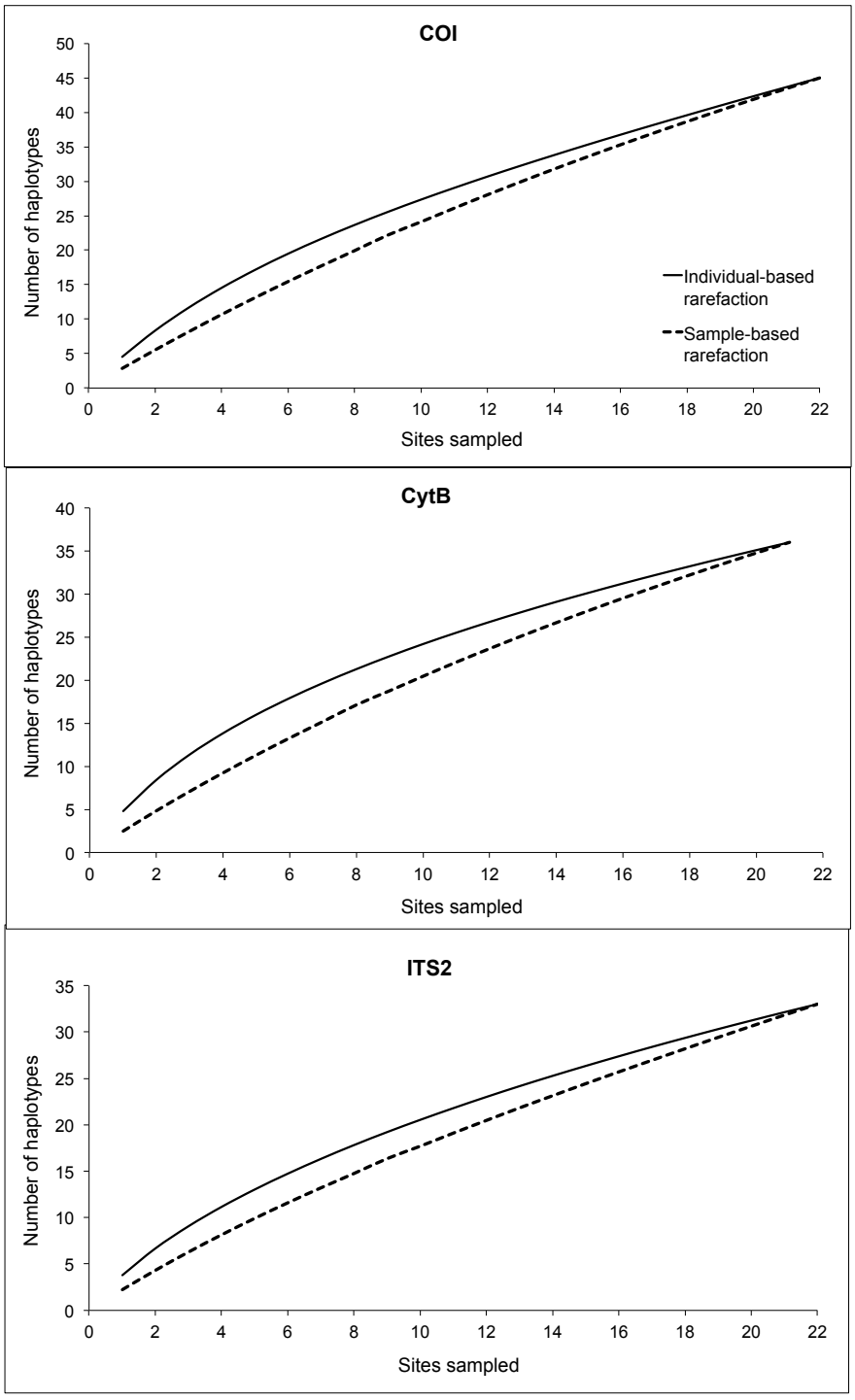

Figure S2.1: Haplotype rarefaction curves for COI, CytB and ITS2 (note the y-axis scale differs for each DNA region). Curves show the accumulation of haplotypes across all cryptic species with increasing number of individuals sequenced (individual-based curves), and increasing number of geographic sites sampled (sample-based curves). None of the curves reach an asymptotic shape, indicating that increased sampling would uncover more haplotypes. Although the two types of rarefaction share a close resemblance, sample-based curves always lie below individual-based curves, reflecting some degree of aggregation of haplotypes within geographic sites.

Table S2.1: Sampling location and site details, with corresponding number of *Eusirus* specimens collected or donated (both morphological species combined).

Location	Site			Research Vessel	Voyage station reference	Depth range (m)	Coordinates	*Eusirus* (n)
Tressler Bank (TB)	TBa	pTBD	gTBD	*Aurora Australis*	V2 2009/10 BTC 11	597 - 698	64°16'57.47"S, 97° 7'10.38"E	2
	TBb	pTB1-3	gTB1-2		V2 2009/10 BTC 20	435 - 449	64°48' 3.60"S, 94°11'31.13"E	5
	TBc	pTBW11-15	gTBW4-7		V2 2009/10 BTC 26	393 - 413	65°52'28.38"S, 89°17'11.88"E	9
	TBd	pTBW1-10	gTBW1-3		V2 2009/10 BTC 30	502 - 547	65°50'3.84"S, 89°32'38.40"E	13
							Total	**29**
East Coast (EC)	ECa	pECO		*Aurora Australis*	V3 2007/08 CEAMARC 79	420 - 668	66°34'49.26"S, 143° 5'14.28"E	20
	ECb	pECS			V3 2007/08 CEAMARC 43	163 - 361	65°42'24.93"S, 140°35'50.59"E	6
	ECc	gEC			V3 2007/08 CEAMARC 8	365 - 386	66°45'57.40"S, 143°24'28.67"E	15
	ECd	gECE			V3 2007/08 CEAMARC 16	510 - 626	66°33'25.41"S, 142°16'38.10"E	11
	ECe	gECD			V3 2007/08 CEAMARC 39	862 - 875	66°20'46.57"S, 140° 1'50.00"E	3
							Total	**55**
Ross Sea (RS)	RSa	pRSC		*Tangaroa*	TAN 0802/ 94 & 100*	445 - 459	76°12' 7.20"S, 176°14'52.80"E	3
	RSb	pRSE			TAN 0802/ 161	535 - 536	72° 4'31.80"S, 172°54'15.48"E	8
	RSc	gRS			TAN 0802/ 56	528 - 531	75°37'58.80"S, 169°51' 0.00"E	6
	RSd	gRSI			TAN 0802/ 70	724 - 752	76°46'30.00"S, 169°50'60.00"E	4
	RSe	gRSD			TAN 0802/ 41	916 - 930	74°43'34.32"S, 167° 0'47.52"E	1
							Total	**22**
Antarctic Peninsula (PN)	PNa	pEI		*Polarstern*	ANT-XIX/3 PS61/ ?	unknown	Elephant Island (61°S, 55°W)	2
	PNb	pSI			ANT-XIX/3 PS61/ 103-1	256 - 296	61°44'52.80"S, 58° 1'32.40"W	1
	PNc	pPN			ANT-XXIII/8 PS69/ 721-2	295 - 299	65°55'24.60"S, 60°34' 0.60"W	2
	PNd	gPen			ANT-XXIII/8 PS69/ 700-2	442 - 445	65°55' 4.20"S, 60°20' 9.00"W	2
							Total	**7**
Weddell Sea (WD)	WDa	pWD		*Polarstern*	ANT-XXI/2 PS65/ 253-1	295 - 309	71° 4'53.40"S, 11°32'12.60"W	4
	WDb	pWDW	gWDW		ANT-XXIV/2 PS71/ 048-1	595 - 602	70°23'53.40"S, 8°18'40.20"W	6
	WDc	pWDE1-2			ANT-XXI/2 PS65/ 293-1	518 - 542	72°51'54.00"S, 19°39'18.60"W	2
	WDd	pWDE3-4			ANT-XXI/2 PS65/ 308-1 & 326-1*	616 - 622	72°51'25.80"S, 19°38'40.20"W	2
							Total	**14**

* Voyage stations separated by less than 3km were treated as a single site, as individual trawls themselves often covered a similar area.

APPENDIX II

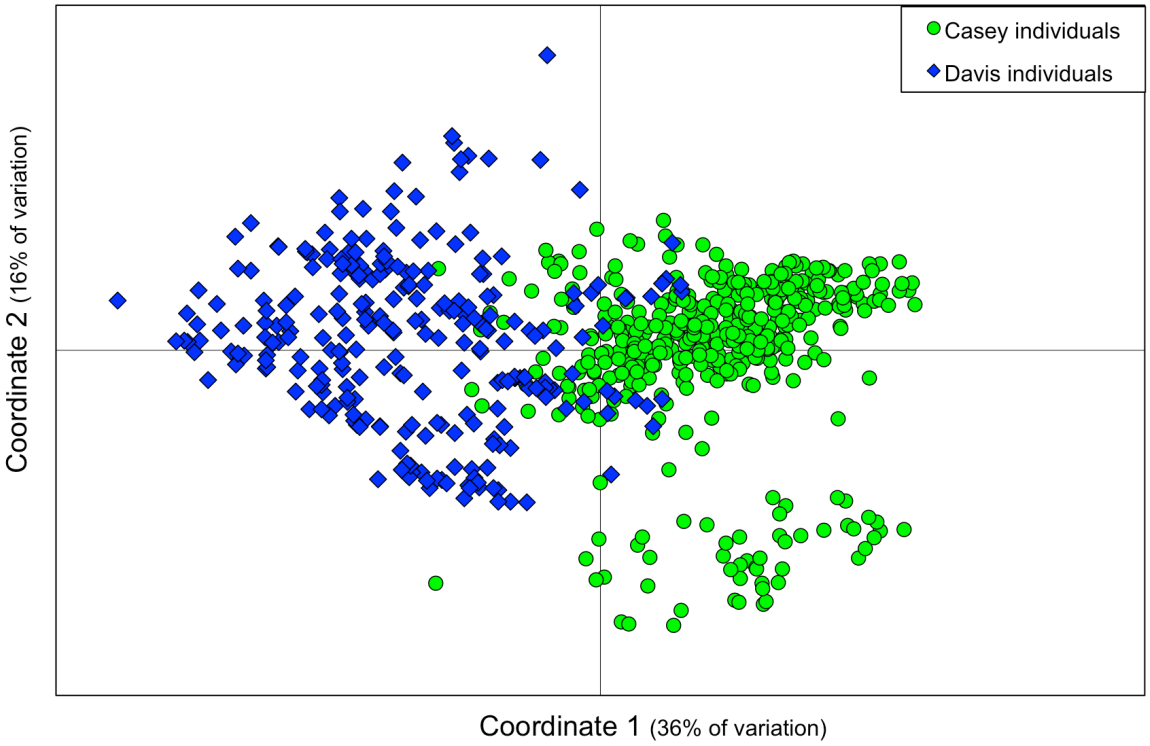

Figure S3.1: Results of Principal Coordinate Analysis on multilocus genotypes of *O. franklini*. Over 50% of genetic variation is explained within the first two coordinates, however, there is no single group sufficiently discrete to indicate cryptic species in the dataset.

Table S3.1: Regions, locations, and sites sampled for *O. franklini*, with corresponding sample size (*n*). Locations (and respective sites) in bold have been classified as polluted.

Region	Location (code)	Site	*n*
Casey	Honkala (HO)	HO	30
	McGrady (MG)	MGa	19
		MGb	32
	Peterson (PE)	PEa	30
		PEb	31
	Sparkes (SP)	SPa	26
		SPb	30
	Brown (BB)	**BBa**	30
		BBb	30
		BBc	30
		BBd	14
	Newcombe (NE)	**NEa**	30
		NEb	30
	Shannon (SH)	**SHa**	30
		SHb	30
	Wilkes (WK)	**WK**	26
		Total for Casey:	*448*
Davis	Old Wallow (OW)	OWa	30
		OWb	30
	Sorsdal (SD)	SDa	30
		SDb	30
		SDc	30
	Zappet (ZP)	ZPa	30
		ZPb	30
	Wharf (WH)	**WHa**	30
		WHb	30
		Total for Davis:	*270*

Table S3.2: Inbreeding coefficients by locus (F_{IS}) and the number of private alleles (P_A) for each population of *O. franklini*. Asterisks indicate significant departures from Hardy-Weinberg Equilibrium ($p<0.05$) after Bonferroni correction. Dashes indicate that a locus was monomorphic, hence F_{IS} could not be estimated. Polluted sites are in bold.

			F_{IS}						
		P_A	*Orcfra3*	*Orcfra4*	*Orcfra5*	*Orcfra6*	*Orcfra12*	*Orcfra13*	*Orcfra26*
Casey	HO	2	0.141	0.791	0.659	0.072	0.117	-0.394	-0.047
	MGa	0	0.004	0	-0.015	-0.042	-0.011	0.129	-0.029
	MGb	0	0.043	-0.034	-0.018	0.187	0.008	0.021	-0.008
	PEa	1	-0.216	-0.010	-0.020	0.037	0.018	0.056	---
	PEb	0	0.019	-0.008	-0.018	0.046	-0.054	0.142	---
	SPa	0	-0.096	-0.042	0.653	0.057	0.147	0.031	0
	SPb	1	0.162	0.458	0	0.131	0.014	0.010	-0.009
	BBa	0	0.162	-0.018	0.477	-0.091	0.103	-0.037	---
	BBb	0	0.105	-0.020	-0.020	-0.013	0.024	-0.092	---
	BBc	0	-0.014	-0.009	-0.020	0.035	-0.073	0.172	---
	BBd	0	-0.044	0	---	0.142	0.265	0.524	0
	NEa	0	0.077	-0.036	-0.059	-0.153	0.158	-0.607*	0
	NEb	0	0.042	0.728	---	-0.001	0.051	0.113	0
	SHa	1	-0.074	-0.009	---	-0.071	-0.023	-0.040	0
	SHb	0	0.134	0.220	0.786	0.114	0.122	0.161	---
	WK	0	-0.015	-0.029	1.000	-0.080	0.068	-0.308	0
Davis	OWa	3	0.132	0.604*	0.419	-0.106	-0.072	-0.020	-0.009
	OWb	0	0.081	0.827*	0.292	0.173	-0.020	-0.072	0.192
	SDa	1	-0.085	0.587*	0.454	0.203	0.044	0.251	0.163
	SDb	2	-0.254	0.679*	0.190	-0.123	0.091	0.095	0.139
	SDc	3	-0.043	0.594*	0.377	0.232	-0.037	-0.127	-0.148
	ZPa	1	0.158	0.421	0.794	0.009	0.043	-0.210	-0.028
	ZPb	1	0.172	0.893*	-0.018	0.012	-0.023	-0.040	-0.182
	WHa	0	0.029	0.425	-0.049	0.423	-0.025	-0.095	0.348
	WHb	0	0.106	0.736*	0.663	0.023	-0.059	-0.128	-0.226

Table S3.3: Matrix of pairwise differentiation estimates for all *O. franklini* populations sampled at Casey and Davis. F_{ST} below diagonal; R_{ST} above diagonal. Estimates of differentiation *between* Casey and Davis populations are italicized. Negative values have been converted to zero. Polluted sites are in bold.

	HO	MGa	MGb	PEa	PEb	SPa	SPb	**BBa**	**BBb**	**BBc**	**BBd**	**NEa**	**NEb**	**SHa**	**SHb**	**WK**	OWa	OWb	SDa	SDb	SDc	ZPa	ZPb	**WHa**	**WHb**
HO	-	0.002	0.010	0.106	0.036	0.013	0.030	0.000	0.013	0.000	0.012	0.048	0.015	0.000	0.000	0.000	*0.202*	*0.309*	*0.138*	*0.129*	*0.161*	*0.362*	*0.321*	*0.216*	*0.124*
MGa	0.001	-	0.000	0.062	0.003	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	*0.170*	*0.287*	*0.095*	*0.087*	*0.122*	*0.350*	*0.309*	*0.197*	*0.086*
MGb	0.005	0.000	-	0.039	0.000	0.000	0.000	0.002	0.015	0.000	0.010	0.019	0.000	0.000	0.006	0.005	*0.223*	*0.340*	*0.145*	*0.134*	*0.174*	*0.400*	*0.357*	*0.253*	*0.134*
PEa	0.011	0.015	0.012	-	0.000	0.035	0.034	0.100	0.119	0.058	0.109	0.087	0.029	0.074	0.111	0.090	*0.314*	*0.435*	*0.219*	*0.203*	*0.251*	*0.486*	*0.452*	*0.345*	*0.207*
PEb	0.014	0.010	0.005	0.007	-	0.000	0.000	0.026	0.035	0.003	0.024	0.022	0.000	0.017	0.035	0.019	*0.228*	*0.341*	*0.146*	*0.137*	*0.180*	*0.399*	*0.355*	*0.264*	*0.137*
SPa	0.012	0.000	0.000	0.006	0.010	-	0.000	0.001	0.007	0.000	0.001	0.007	0.000	0.000	0.009	0.000	*0.202*	*0.321*	*0.120*	*0.111*	*0.152*	*0.380*	*0.337*	*0.233*	*0.112*
SPb	0.023	0.007	0.000	0.031	0.013	0.001	-	0.012	0.019	0.000	0.006	0.013	0.005	0.008	0.024	0.009	*0.231*	*0.358*	*0.141*	*0.129*	*0.173*	*0.418*	*0.377*	*0.264*	*0.133*
BBa	0.000	0.000	0.000	0.009	0.001	0.000	0.010	-	0.000	0.000	0.000	0.010	0.009	0.000	0.000	0.000	*0.173*	*0.279*	*0.105*	*0.100*	*0.132*	*0.340*	*0.293*	*0.198*	*0.098*
BBb	0.004	0.000	0.002	0.007	0.001	0.000	0.009	0.000	-	0.000	0.000	0.000	0.020	0.000	0.000	0.000	*0.153*	*0.263*	*0.083*	*0.080*	*0.112*	*0.330*	*0.278*	*0.185*	*0.078*
BBc	0.002	0.000	0.000	0.014	0.004	0.006	0.011	0.000	0.001	-	0.000	0.010	0.000	0.000	0.000	0.000	*0.198*	*0.309*	*0.122*	*0.115*	*0.152*	*0.368*	*0.322*	*0.226*	*0.114*
BBd	0.008	0.001	0.000	0.026	0.002	0.010	0.000	0.000	0.001	0.000	-	0.014	0.034	0.010	0.000	0.000	*0.143*	*0.252*	*0.072*	*0.063*	*0.097*	*0.309*	*0.270*	*0.164*	*0.072*
NEa	0.025	0.000	0.006	0.013	0.009	0.001	0.016	0.002	0.001	0.007	0.010	-	0.018	0.014	0.023	0.017	*0.152*	*0.267*	*0.074*	*0.071*	*0.106*	*0.341*	*0.286*	*0.195*	*0.070*
NEb	0.007	0.000	0.010	0.013	0.005	0.009	0.026	0.000	0.002	0.004	0.018	0.010	-	0.000	0.010	0.013	*0.214*	*0.326*	*0.139*	*0.132*	*0.169*	*0.386*	*0.343*	*0.244*	*0.126*
SHa	0.009	0.000	0.000	0.019	0.006	0.008	0.017	0.000	0.009	0.000	0.001	0.006	0.001	-	0.000	0.000	*0.184*	*0.291*	*0.118*	*0.112*	*0.144*	*0.351*	*0.307*	*0.211*	*0.108*
SHb	0.000	0.000	0.000	0.019	0.007	0.005	0.006	0.000	0.000	0.000	0.000	0.007	0.001	0.000	-	0.000	*0.179*	*0.284*	*0.115*	*0.110*	*0.140*	*0.343*	*0.298*	*0.201*	*0.106*
WK	0.002	0.000	0.002	0.009	0.005	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	*0.176*	*0.285*	*0.105*	*0.098*	*0.133*	*0.343*	*0.298*	*0.199*	*0.095*
OWa	*0.151*	*0.153*	*0.166*	*0.164*	*0.148*	*0.169*	*0.165*	*0.152*	*0.143*	*0.168*	*0.144*	*0.137*	*0.138*	*0.167*	*0.147*	*0.141*	-	0.017	0.000	0.000	0.000	0.071	0.033	0.002	0.003
OWb	*0.136*	*0.140*	*0.156*	*0.144*	*0.129*	*0.153*	*0.160*	*0.137*	*0.124*	*0.155*	*0.134*	*0.121*	*0.120*	*0.155*	*0.140*	*0.125*	0.000	-	0.048	0.050	0.018	0.004	0.000	0.000	0.064
SDa	*0.150*	*0.155*	*0.172*	*0.152*	*0.141*	*0.166*	*0.171*	*0.150*	*0.136*	*0.169*	*0.154*	*0.131*	*0.132*	*0.170*	*0.152*	*0.141*	0.000	0.000	-	0.000	0.000	0.109	0.061	0.032	0.000
SDb	*0.147*	*0.150*	*0.163*	*0.150*	*0.138*	*0.160*	*0.163*	*0.146*	*0.134*	*0.163*	*0.144*	*0.127*	*0.131*	*0.162*	*0.146*	*0.134*	0.000	0.000	0.000	-	0.000	0.108	0.064	0.029	0.000
SDc	*0.181*	*0.188*	*0.196*	*0.185*	*0.173*	*0.197*	*0.196*	*0.180*	*0.169*	*0.199*	*0.182*	*0.162*	*0.165*	*0.198*	*0.179*	*0.173*	0.000	0.006	0.000	0.000	-	0.070	0.032	0.008	0.000
ZPa	*0.145*	*0.155*	*0.169*	*0.153*	*0.143*	*0.164*	*0.167*	*0.154*	*0.136*	*0.170*	*0.153*	*0.136*	*0.132*	*0.169*	*0.148*	*0.134*	0.010	0.003	0.006	0.006	0.020	-	0.003	0.022	0.129
ZPb	*0.141*	*0.152*	*0.163*	*0.144*	*0.129*	*0.158*	*0.169*	*0.146*	*0.126*	*0.162*	*0.143*	*0.124*	*0.127*	*0.162*	*0.149*	*0.129*	0.022	0.000	0.011	0.016	0.030	0.004	-	0.000	0.078
WHa	*0.141*	*0.146*	*0.163*	*0.154*	*0.140*	*0.164*	*0.167*	*0.151*	*0.135*	*0.166*	*0.143*	*0.134*	*0.132*	*0.163*	*0.149*	*0.136*	0.005	0.000	0.012	0.008	0.023	0.008	0.003	-	0.034
WHb	*0.150*	*0.158*	*0.176*	*0.159*	*0.149*	*0.174*	*0.182*	*0.158*	*0.145*	*0.174*	*0.158*	*0.138*	*0.136*	*0.173*	*0.158*	*0.140*	0.003	0.000	0.000	0.001	0.006	0.003	0.002	0.001	-

APPENDIX III

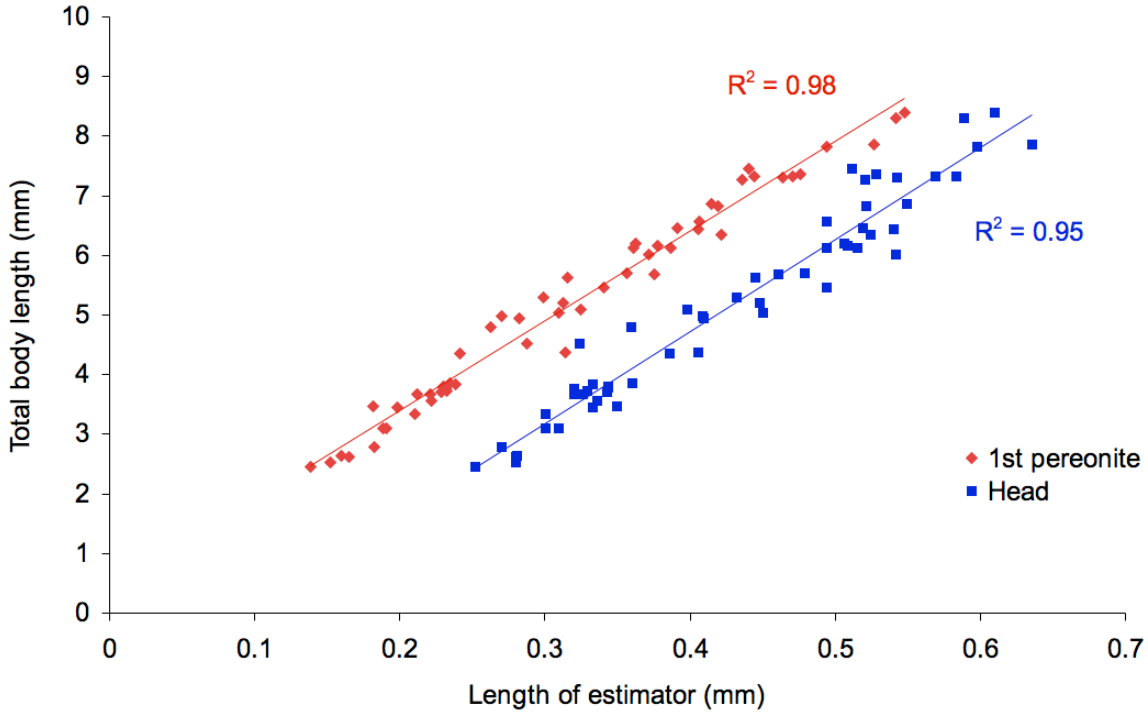

Figure S4.1: Linear regression of the two potential total length estimators – dorsal length of the head and dorsal length of the first pereonite (n = 55) – in *O. franklini*. The latter was shown to have the strongest correlation to total body length ($R^2 = 0.98$, $p < 0.001$).

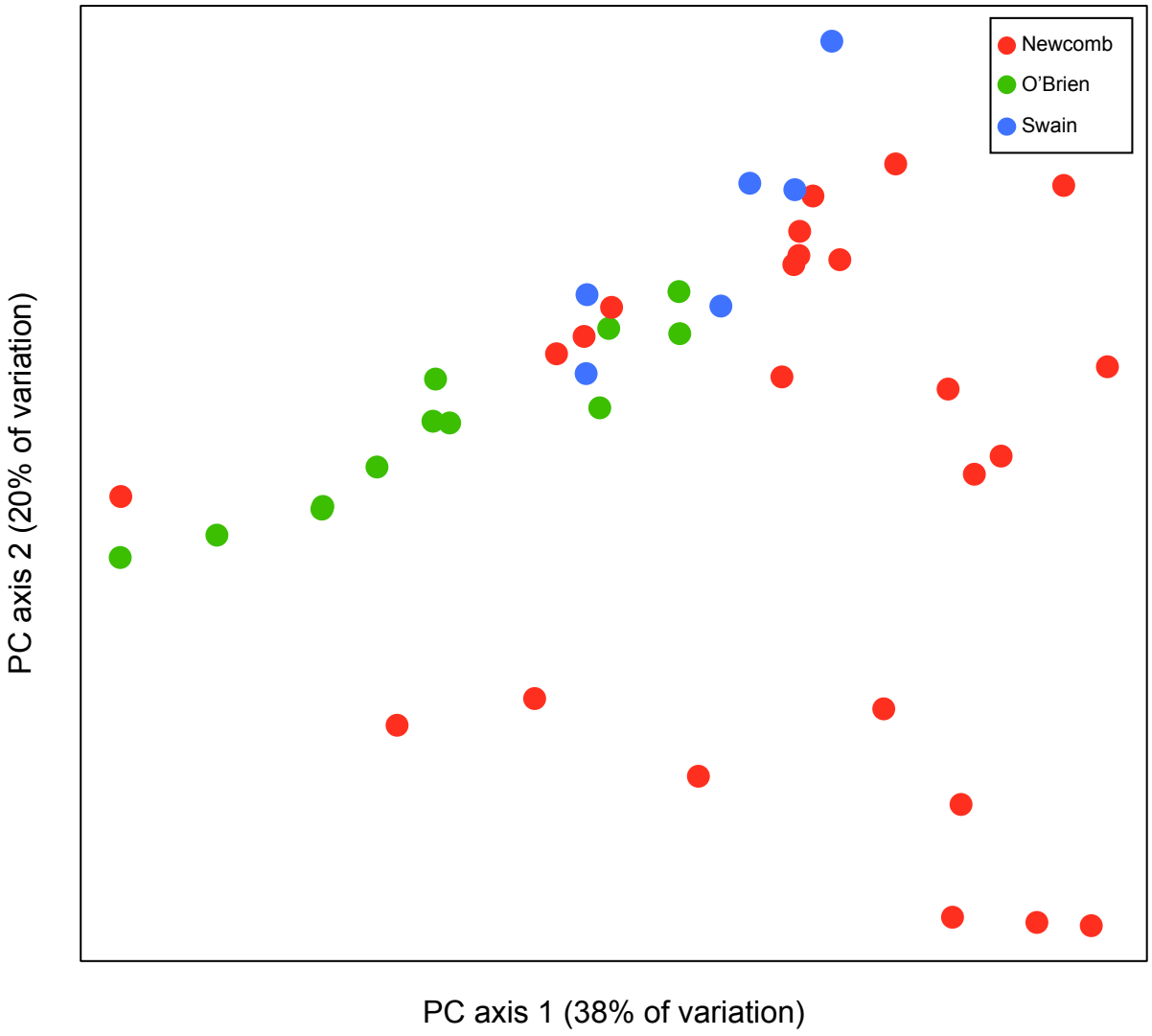

Figure S4.2: PCA ordination of all sediment parameters at Casey. The first two principal components account for 58% of the total environmental variation. Points are coloured by geographic area. Areas are significantly different with respect to the nature of their sediments ($R = 0.21$, $p < 0.01$).

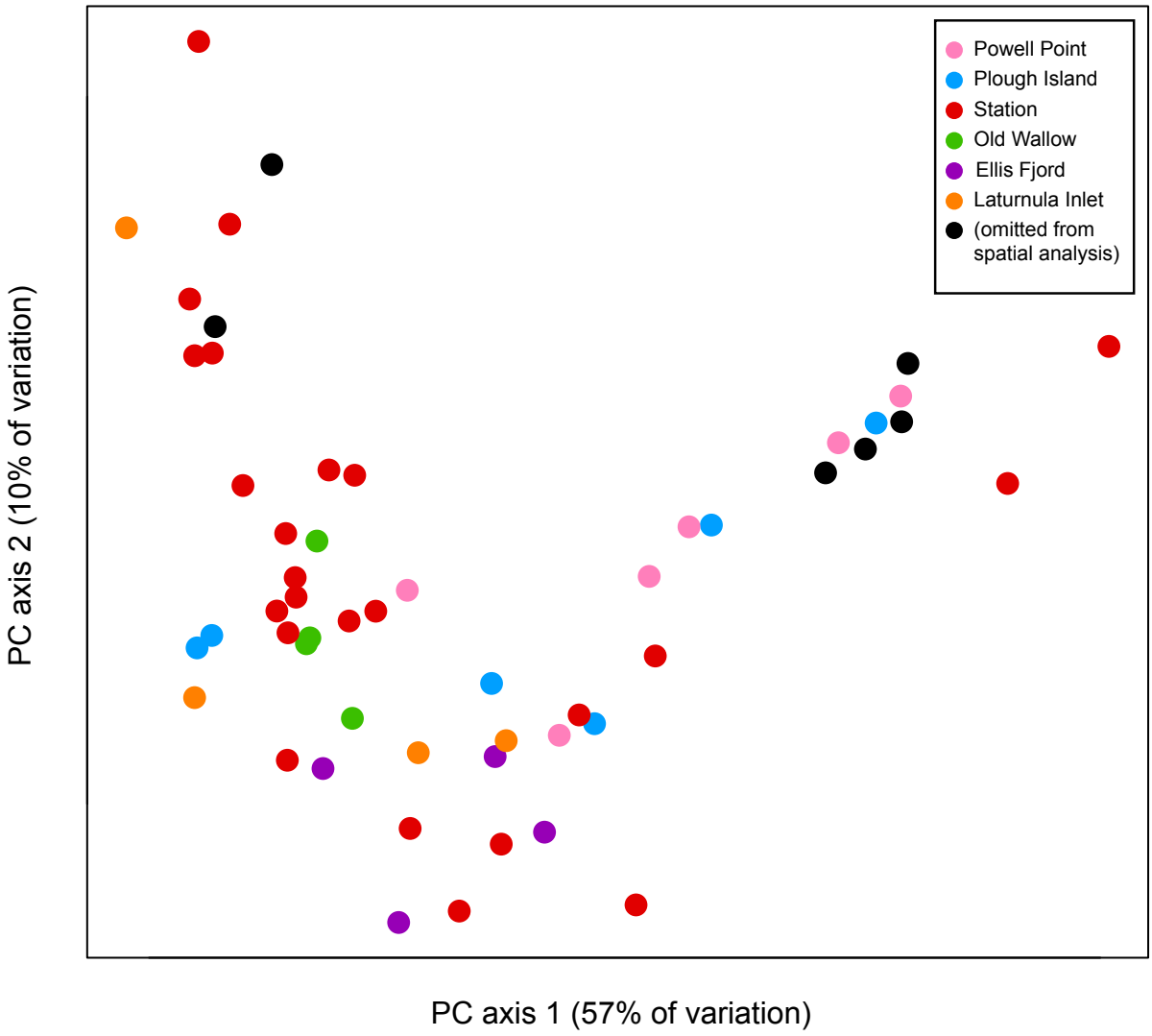

Figure S4.3: PCA ordination of all sediment parameters at Davis. The first two principal components account for 67% of the total environmental variation. Points are coloured by geographic area. Areas are not significantly different with respect to the nature of their sediments ($R = 0.14$, $p = 0.06$).

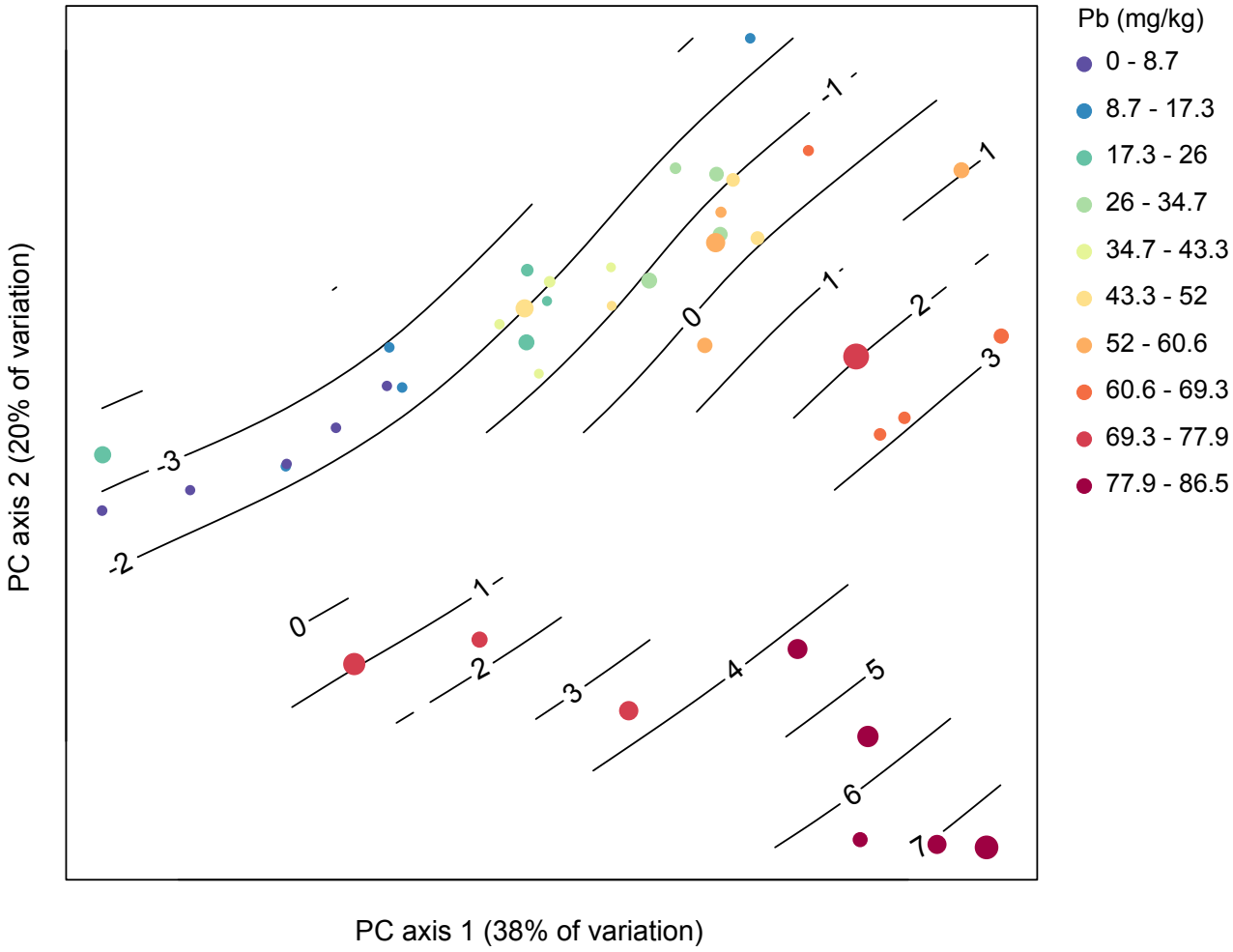

Figure S4.4: PCA ordination of all sediment parameters for Casey. The first two principal components account for 58% of environmental variation. Points are scaled by the mean abundance of *O. franklini* and coloured by lead concentration (increasing from cool to warm colours). Contours represent predictions of square-root transformed abundance based on GAM (note that negative contours are the result of smoothing by GAM). The concentration of most trace metals increases from the top left to bottom right of the PCA, as do the predictions of abundance.

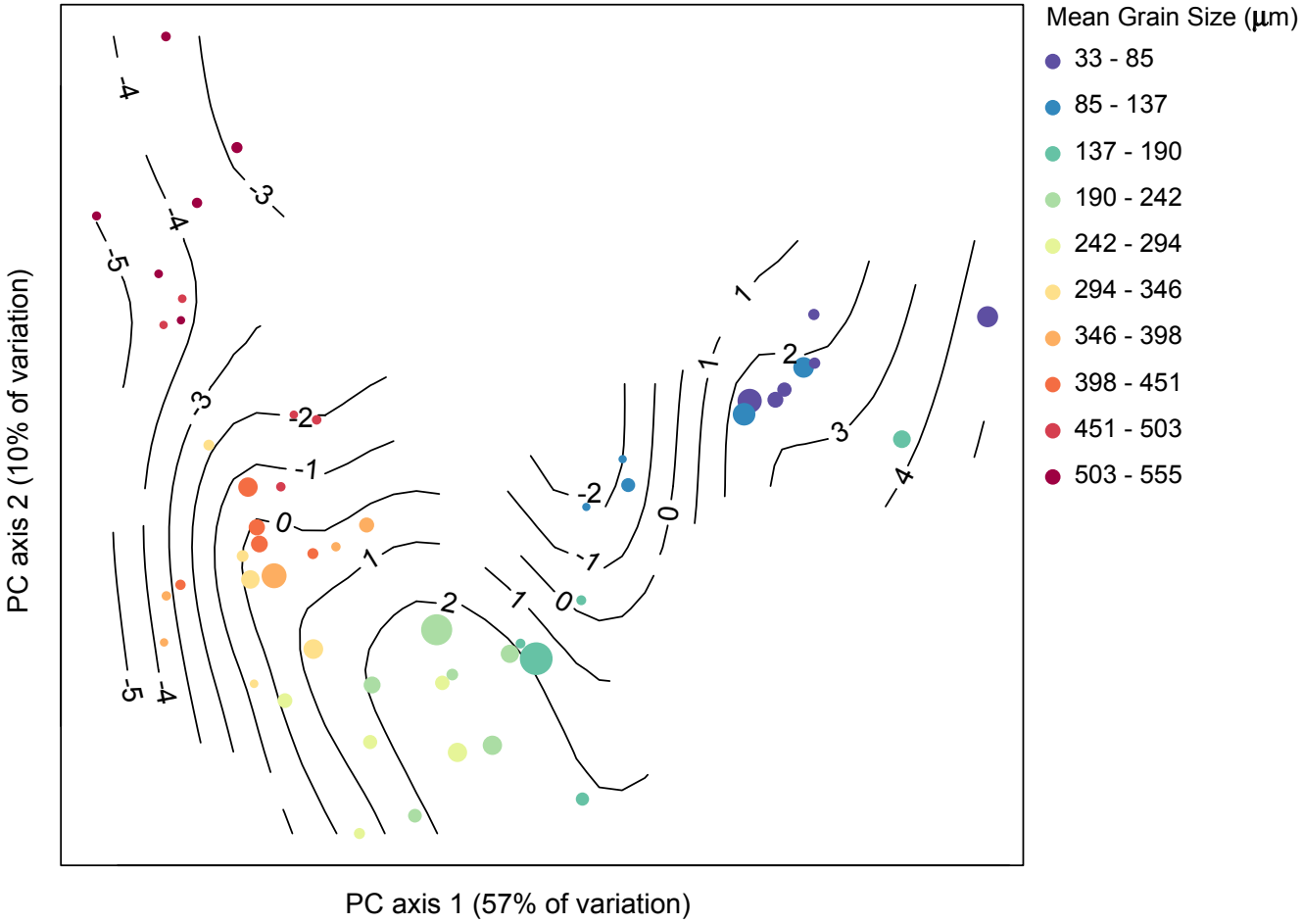

Figure S4.5: PCA ordination of sediment parameters for Davis. The first two principal components account for 67% of environmental variation. Points are scaled by the mean abundance of *O. franklini* and coloured by mean grain size (increasing from cool to warm colours). Contours represent predictions of square-root transformed abundance based on GAM (note that negative contours are the result of smoothing by GAM). Overall there is a predicted increase in abundance with decreasing mean grain size.

Table S4.1: Sediment parameters used to analyse the distribution of *O. franklini.*

		Description
Grain size parameters	Clay/silt	Proportion sediment grains < 63µm
	Very fine sand	Proportion sediment grains 63µm - 125µm
	Fine sand	Proportion sediment grains 125µm - 250µm
	Medium sand	Proportion sediment grains 250µm - 500µm
	Coarse sand	Proportion sediment grains 500µm - 1mm
	Very coarse sand	Proportion sediment grains 1mm - 2mm
	Other sediments	Proportion sediment grains > 2mm
	Mean grain size	Mean size of sediment grains measured in µm
Trace element parameters*	Al	Aluminium
	As	Arsenic
	Ba	Barium
	Cd	Cadmium
	Co	Cobalt
	Cr	Chromium
	Cu	Copper
	Fe	Iron
	Mg	Magnesium
	Mo	Molybdenum
	Ni	Nickel
	Pb	Lead
	S	Sulfur
	Sb	Antimony
	Sn	Tin
	Sr	Strontium
	V	Vanadium
	Zn	Zinc
	TOC	Total organic carbon as % total sediment mass

* All trace elements were measured as mg/kg present in the < 2mm fraction of sediment.

APPENDIX IV

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Baird HP, Miller KJ and Stark JS (2010) Evidence of hidden biodiversity, ongoing speciation and diverse patterns of genetic structure in giant Antarctic amphipods. *Molecular Ecology* **20**, 3439-3454.